JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXX WASHINGTON, D. C., MARCH 1, 1925 No. 5

A COMPARISON OF DIRECT AND INDIRECT CALORIMETRY IN INVESTIGATIONS WITH CATTLE[1]

By MAX KRISS

Associate in Animal Nitrition, Institute of Animal Nutrition of Pennsylvania State College

INTRODUCTION

From the time of the construction of the first respiration calorimeter by Lavoisier (*9, p. 355*)[2] until Rubner (*10 p. 185*) demonstrated the applicability of the law of conservation of energy to the vital processes of the animal body, the interest of experimenters with animal calorimeters centered mainly in the question as to whether the products of respiration fully represent the production of heat by the animal.

With the establishment of the validity of this conception began a new era in respiration calorimetry, which has been marked by the perfecting of apparatus and the standardization of methods. Since, however, the respiration calorimeter is a complicated and costly apparatus, both in construction and use, methods have been evolved for the indirect determination of the heat production, by the use of a respiration apparatus, without the calorimetric features.

For the purpose of indirect heat estimation there are now in use two principal methods, with modifications of detail, to meet special conditions as to kind of animal, length of experiment, equipment available, accuracy sought, and the particular problem in hand.

Briefly and generally, one of these methods (*2*) involves the estimation of the amounts of protein, carbohydrates, and fat oxidized in the body, and the computation of the heat production from the calorific values of these nutrients.

The amounts katabolized are computed from the oxygen consumed and carbon dioxide produced, and the nitrogen, carbon, oxygen, and hydrogen of the urine, these computations being based on the respiratory quotients (proportions of carbon dioxide produced to oxygen consumed) for the nutrients oxidized, these quotients being approximately 0.7 for fat, 0.8 for protein and 1.0 for carbohydrates.

The other method depends on the balances of nitrogen and carbon in the animal body, and the determination of the potential energy of the feed and excreta. By this method the heat production is measured by deducting from the gross energy of the feed the potential energy of the excreta and the energy equivalent of the body tissue gained (or adding the energy equivalent of tissue lost).

The more fundamental considerations as to energy metabolism having been established, a question of much importance is the comparative accuracy of the expensive and time-consuming direct-heat estimation by means of the respiration calorimeter and the relatively simple indirect-heat estimation by means of the two general procedures outlined.

As a contribution to the understanding of this problem the writer presents the following consideration of the possibilities of error in each, and the extent to which these errors affect results, the comparisons being based on the experimental work of this institute.

The respiration calorimeter at the Institute of Animal Nutrition, which has been used for more than 20 years in metabolism experiments with cattle, is, as the name implies, both a calorimeter and a respiration apparatus. In each experiment heat production has been determined directly, and also, for comparison, by computation from the balance of matter and energy, as in the second of the indirect methods referred to above. This method is essentially the same as that used by Kellner (*7*) in his extensive researches on cattle.

[1] Received for publication June 11, 1924; issued May, 1925. This study was undertaken at the suggestion of Dr. E. B. Forbes, to whom the writer is especially indebted for kindly criticism and assistance in revising the manuscript. He is also under obligations to the other members of the staff of the Institute for many valuable suggestions in connection with this work.

[2] Reference is made by number (italic) to "Literature cited," p. 406.

A comparison of the observed and computed heat production in experiments with steers, conducted in the years prior to and including 1909, has been reported by Armsby (*6*). Since the year 1909 a number of experiments have been made on steers, and on cows as well, and upon these the present paper is based.

OBSERVED AND COMPUTED HEAT PRODUCTION

Before entering upon a discussion of the direct and indirect methods of determining the heat production by animals let us compare the results obtained by the two methods. Tables I and II present the results of experiments with steers and cows, the steer experiments comprising 35 periods and the cow experiments 36. Each period was of 48 hours' duration (except in experiment 221a and 221d, 886–I, where the periods were 24 hours).[3] All results have been computed to a 24-hour basis.

The results of the steer experiments show that in 21 cases out of the 35 the computed heat production is higher than the observed; in 10 cases the computed heat is lower than the observed, and in 4 cases the difference between the two is so small as to be negligible.

Considering the differences in the individual trials, and arranging them in groups according to magnitude, the results are as follows: In 21 cases, or 60.0 per cent of the total number, the difference exceeds 1 per cent, of which 13 are plus (that is, show the computed heat production the larger); in 14 cases, or 40.0 per cent of the total number, the difference exceeds 2 per cent, of which 10 are plus; in 6 cases, or 17.1 per cent of the total number, the difference exceeds 3 per cent, of which 5 are plus; in 2 cases, or 5.7 per cent of the total number, the difference exceeds 4 per cent, of which both are plus; and in 2 cases, or 5.7 per cent of the total number, the difference exceeds 5 per cent, of which both are plus.

TABLE I.—*Observed and computed heat production of steers*

Experiment No.	Animal	Period No.	Gain by animal	Heat production in 24 hours		Difference	
				Computed	Observed		
			Calories	*Calories*	*Calories*	*Calories*	*Per cent*
210	Steer D	I	−845.7	9,262.9	9,460.6	−197.7	−2.1
		II	−1,141.5	8,026.1	8,186.0	−159.9	−1.9
		III	−2,153.8	6,942.0	7,110.4	−168.4	−2.4
211	do	I	−883.2	12,139.1	11,546.9	+592.2	+5.1
		II	−496.9	9,813.8	9,596.5	+217.3	+2.3
		III	+7,663.8	13,815.6	13,937.2	−121.6	−0.9
		IV	−2,462.1	9,227.4	9,196.5	+30.9	+0.3
		V	−4,968.6	8,010.9	7,952.9	+58.0	+0.7
211	Steer G	I	+327.9	11,642.1	11,710.8	−68.7	−0.6
		II	−1,565.5	8,229.9	8,197.0	+32.9	+0.4
		III	+6,133.6	12,807.3	13,290.2	−482.9	−3.6
		IV	−2,981.5	8,942.6	8,936.5	+6.1	+0.1
		V	−4,154.1	6,894.8	6,882.5	+12.3	+0.2
212	Steer H	I	+2,241.2	11,130.7	11,022.9	+107.8	+1.0
		II	+2,529.3	10,690.4	10,903.4	−213.0	−1.9
		III	+840.4	9,712.9	9,723.1	−10.2	0.1
		IV	+859.9	9,805.8	9,701.6	+104.2	+1.1
		V	−739.6	7,296.0	7,304.9	−8.9	−0.1
		VI	−409.8	6,950.4	6,773.1	+177.3	+2.6
216	Steer J	I	+5,111.5	15,931.6	15,541.2	+390.4	+2.5
		II	−1,046.5	9,836.3	9,547.2	+289.1	+3.0
		III	+596.7	11,420.2	10,865.8	+554.4	+5.1
		IV	−2,672.9	8,186.4	8,020.4	+166.0	+2.1
		V	+2,299.7	12,820.9	12,827.5	−6.6	0.1
		VI	+907.3	11,062.8	10,838.2	+224.6	+2.1
		VII	−1,708.8	8,501.5	8,398.4	+103.1	+1.2
217	do	I	+927.2	11,240.9	10,896.8	+344.1	+3.2
		II	+8,493.1	16,934.8	16,671.7	+263.1	+1.6
		III	+6,463.3	21,416.0	21,204.9	+211.1	+1.0
		IV	−559.6	14,716.0	14,224.4	+491.6	+3.5
220	Steer K	I	−626.0	11,881.5	12,123.6	−242.1	−2.0
		II	−2,270.5	10,108.7	10,288.1	−179.4	−1.7
		III	+464.5	10,809.7	10,754.2	+55.5	+0.5
		IV	+4,644.5	13,524.3	13,844.0	−319.7	−2.3
		V	−2,951.5	10,136.3	10,055.8	+80.5	+0.8
Totals and averages, 35 periods				379,868.6	377,535.2	+2,333.4	+0.6

[3] Period 886–I, experiment 221d, was to have covered 48 hours, but owing to the failure of the electric current the experiment was discontinued during the second 24 hours.

TABLE II.—*Observed and computed heat production of cows*

Experiment No.	Cow No.	Period No.	Dry or in milk	Gain by animal	Heat production in 24 hours		Difference	
					Computed	Observed		
				Calories	*Calories*	*Calories*	*Calories*	*Per cent*
221a	631	I	In milk	+221.4	11,720.5	11,463.9	+256.6	+2.2
		II	do	+3,663.2	12,598.8	12,333.9	+264.9	+2.2
		III	Dry	+3,863.5	13,680.3	13,219.2	+461.1	+3.5
221a	615	I	In milk	+502.0	11,756.5	11,435.5	+321.0	+2.8
		II	do	−160.7	12,286.2	12,137.3	+148.9	+1.2
		III	do	−650.3	12,747.6	12,032.2	+715.4	+6.0
221a	579	I	do	+384.7	13,545.9	13,235.6	+310.3	+2.3
		II	do	+686.7	13,165.5	12,896.2	+269.3	+2.1
221c	885	I	do	−2,883.6	11,007.1	11,221.4	−214.3	−1.9
		II	do	−2,957.5	9,023.7	8,862.4	+161.3	+1.8
		III	do	−4,830.4	9,032.1	8,859.0	+173.1	+2.0
221c	886	I	do	+2,976.8	10,183.6	11,191.7	−1,008.1	−9.0
		II	do	−1,228.2	8,228.7	8,568.6	−339.9	−4.0
		III	do	+3,018.2	10,885.9	11,063.4	−177.5	−1.6
221d	885	I	Dry	+730.6	8,201.5	8,380.3	−178.8	−2.1
		II	do	+4,410.1	9,099.0	9,629.1	−530.1	−5.5
		III	do	+366.9	9,289.5	8,816.7	+472.8	+5.4
221d	886	I	do	+897.8	8,407.9	8,036.9	+371.0	+4.6
		II	do	+4,389.2	10,774.5	10,474.5	+300.0	+2.9
		III	do	+895.5	8,511.0	7,892.4	+618.6	+7.8
221e	885	I	do	+2,625.6	9,157.4	9,721.7	−564.3	−5.8
		II	do	−155.0	8,444.7	8,716.4	−271.7	−3.1
221e	886	I	In milk	+1,795.0	11,852.0	12,047.9	−195.9	−1.6
		II	do	+1,685.3	11,136.6	11,049.3	+87.3	+0.8
221e	874	I	do	+537.3	11,563.9	11,417.0	+146.9	+1.3
		II	do	+2,998.6	11,456.6	11,512.6	−56.0	−0.5
221f	874	I	Dry	+3,500.6	11,279.2	10,964.2	+315.0	+2.9
		II	do	+159.6	9,445.2	9,076.8	+368.4	+4.1
221f	887	I	do	+2,019.0	10,649.3	10,327.2	+322.1	+3.1
		II	do	+240.0	8,733.5	8,395.0	+338.5	+4.0
221f	886	I	In milk	+26.5	13,187.1	13,589.4	−402.3	−3.0
		II	do	+2,151.0	12,268.1	12,422.0	−153.9	−1.2
221g	887	I	do	+7.0	12,701.3	12,367.7	+333.6	+2.7
		II	do	−5,327.8	8,887.9	8,627.1	+260.8	+3.0
		III	do	−827.5	10,429.9	9,870.3	+559.6	+5.7
		IV	do	+2,270.1	12,010.5	11,670.4	+340.1	+2.9
Totals and averages, 36 periods					387,349.0	383,525.2	+3,823.8	+1.0

The extreme percentage differences are +5.1 and −3.6, while the total computed heat production for the 35 trials exceeds the observed by only 0.6 per cent.

In the 57 earlier steer experiments reported by Armsby (*6*) the total observed heat production for the 57 trials differed from that computed by only 0.4 per cent, while the extreme percentage differences in individual cases were +7.6 and −5.1 per cent. Grouping the results of the 57 trials as above one finds: In 30 cases, or 52.6 per cent of the total number, the difference exceeds 1 per cent, of which 17 are plus (in favor of the computed heat production); in 21 cases, or 36.8 per cent of the total number, the difference exceeds 2 per cent, of which 13 are plus; in 14 cases, or 24.6 per cent of the total number, the difference exceeds 3 per cent, of which 10 are plus; in 6 cases, or 10.5 per cent of the total number, the difference exceeds 4 per cent, of which 4 are plus; in 4 cases, or 7 per cent of the total number, the difference exceeds 5 per cent, of which 3 are plus; in 2 cases, or 3.5 per cent of the total number, the difference exceeds 6 per cent, of which both are plus; and in 1 case, or 1.8 per cent of the total number, the difference exceeds 7 per cent, which is plus.

In general, the results of the steer experiments given in Table I compare favorably with those reported by Armsby in showing a close agreement between the observed and the computed heat production, though there are considerable differences in a few cases.

In the cow experiments the differences between the observed and the computed heat production are wider than with the steers. Of the 36 trials, 24 show a higher computed heat production than the observed, while in 12 cases the observed heat production is higher than that computed, the percentage differences ranging from +7.8 to −9.0. The total computed heat

production for the 36 periods is higher than that observed by 1.0 per cent. Grouping the results as before one has: In 34 cases, or 94.4 per cent of the total number, the difference exceeds 1 per cent, of which 23 are plus; in 26 cases, or 72.2 per cent of the total number, the difference exceeds 2 per cent, of which 20 are plus; in 14 cases, or 38.9 per cent of the total number, the difference exceeds 3 per cent, of which 9 are plus; in 9 cases, or 25 per cent of the total number, the difference exceeds 4 per cent, of which 6 are plus; in 7 cases, or 19.4 per cent of the total number, the difference exceeds 5 per cent, of which 4 are plus; in 2 cases, or 5.6 per cent of the total number, the difference exceeds 6 per cent, of which 1 is plus; in 2 cases, or 5.6 per cent of the total number, the difference exceeds 7 per cent, of which 1 is plus; and in 1 case, or 2.8 per cent of the total number, the difference exceeds 8 per cent which is minus.

In considering, then, the results of Tables I and II, the following questions suggest themselves: First, in comparing the heat production obtained by the direct and the indirect method which of the two possesses the greater accuracy? Second, what are the possible causes for the wider differences between the computed and the observed heat production in the cow experiments than in those made on steers? In studying these questions let us consider, briefly, the sources of error and the methods used in each.

SOURCES OF ERROR IN THE INDIRECT METHOD

The indirect method here considered, by which the figures for computed heat production in Tables I and II were obtained, is the second of the two indirect methods referred to above, the so-called balance method. The basic principle upon which both of these indirect methods are based is that the oxidation of a given substance in the body liberates the same amount of energy as does its oxidation outside the organism. The difference, then, between the gross energy of the feed and the energy of the total excreta, as determined by analytical methods, gives the energy derived from the feed, and is equal to the heat given off by the animal, in case the animal does not gain or lose body tissue. A loss of body tissue, of course, involves a liberation of energy exceeding that derived from the feed; therefore the energy-equivalent of the body tissue lost is added to the energy derived from the feed in computing the total heat production. On the other hand, a gain of body tissue means that not all of the energy derived from the feed was liberated, a part of it being stored in body tissue, the energy-equivalent of which is subtracted from the energy of the feed in computing the heat production.

Representing the heat production by H, the energy of the feed by F, the energy of the total excreta (feces, urine, methane, brushings, and milk) by E, and the energy of the body gain by G, the general working formula for heat production is $H=F-E-G$.

The estimation of the gain or loss of body tissue, which is an essential feature of this method, is computed in accord with the conception of the schematic body, which regards the organic matter of the animal as composed essentially of protein and fat, with at most comparatively small amounts of carbohydrates (glycogen). The supply of glycogen is assumed to remain constant during the experiment, and the gain or loss of protein and fat is estimated from a balance between income and outgo of nitrogen and carbon.

The factors used in the computation are the following: protein$=N\times 6$; fat$=C\times 1.307$; carbon in protein$=$ 52.54 per cent; heat of combustion of protein per gram$=5.7$ Calories; heat of combustion of fat per gram$=9.5$ Calories. The derivation of these factors is fully discussed by Armsby (*2*).

The analytical data needed for computing the heat production by the balance method are: (1) Dry matter in the feed, feces (or feces and urine mixture) and brushings; (2) nitrogen in the feed, feces, urine, milk, and brushings; (3) carbon in feed, feces, urine, milk, and brushings; (4) carbon in the gaseous excreta (respiration apparatus); (5) carbon in methane (by respiration apparatus); (6) energy in the feed, feces, urine, milk, and brushings.

The accuracy of this indirect method obviously depends on the accuracy of the factors used and on the accuracy of the analytical results. To what extent the factors used may be responsible for the differences between the computed and the observed heat production is difficult to say. Certainly any error due to the factors used should be least where the gain or loss of body tissue is least. Referring, however, to the results in Tables I and II, one notices in many cases relatively small differences between the observed and

the computed heat production where the body gains or losses are considerable, and large differences where the gains or losses are comparatively small. It will be noted that in these tables the net gain in calories is given, and that the differences are somewhat obscured in those cases in which there was a gain of one substance and a loss of another by the algebraic addition of their energy equivalents. However, the separate consideration of the gains or losses of protein and fat does not materially alter the general relationship referred to above, since in most cases in which there was a considerable gain or loss of body tissue both fat and protein were gained or lost together.

In those cases, at least, in which the gains or losses of body tissue are small and the differences between the observed and the computed heat production are large, if there is any serious error in the computed heat production it should be looked for in the analytical results. An examination of some of the analytical data recently obtained by the methods used in this institute has revealed possibilities of error which in the case of the experiments with cows deserve especial consideration. In these experiments there is, in addition to the problem of handling the milk, the necessity of collecting urine and feces together, thus giving rise to new difficulties in the handling and preparation of the samples for analysis.

With this exception, essentially the same experimental procedure and analytical methods were followed in the cow experiments as in the steer experiments (*5, p. 200–222*). It is important, however, to bear in mind the following points in connection with the computation of the nitrogen, carbon, and energy balances: (1) In the steer experiments the nitrogen in the feces was determined in the fresh substance, while the carbon was determined in the air-dried sample, by means of a combustion furnace. (2) In the urine the carbon was determined in the fresh material, while the nitrogen was determined both in the fresh material and in a sample dried in the vacuum desiccator in the same manner as for energy determination. From the nitrogen loss thus determined, in drying, an energy correction was computed as applying to the energy determined by the bomb, assuming that the loss of nitrogen in drying represented a loss of urea and a corresponding loss of energy.

In the cow experiments (except in 221a) [4] the feces were collected together with the urine. The nitrogen was determined in the composite of daily samples both in the fresh and in the air-dried materials in all cases, in order to ascertain the loss during drying. Carbon was determined in the air-dried sample by means of the bomb, in all cases, and also (in experiments 221g and 221f) in the fresh substance, by means of an electric combustion furnace. An attempt was made to determine the carbon in the fresh sample of the feces-and-urine mixture in the first of the experiments with cows, but owing to lack of laboratory help during the war and for some time thereafter, and to the initial technical difficulties with the method, no satisfactory determinations of the carbon in the fresh feces-and-urine mixture are available for the earlier cow experiments. The energy of the feces-and-urine mixture was determined in the air-dried sample.

The air-drying was conducted by spreading the material out on trays in a drying closet in which it was exposed to a current of air heated to about 60° C. by passage over a steam coil. To hasten the drying the material was carefully broken up, when partly dried, by means of a spatula. When thoroughly dry, the material (properly protected) was exposed to the air of the grinding room for several days. It was then weighed, ground as rapidly as possible, and preserved for analysis in sealed, glass-stoppered bottles. In this weighing and grinding especially dry or especially damp days were avoided.

LOSS OF NITROGEN AND CARBON IN AIR-DRYING FECES-AND-URINE MIXTURE

A comparison of determinations of the daily nitrogen and carbon of the feces-and-urine mixture, in the fresh samples, with determinations on air-dried samples, revealed the fact that a considerable loss of these constituents occurred during the process of drying, and that the loss of carbon exceeded the quantity required to combine with the nitrogen lost to form either urea or ammonium carbonate. Table III exhibits these losses as occurring in experiments 221f and 221g.

The figures in Table III show clearly that the carbon in the air-dried feces and urine is far from the original total, even after adding to it the carbon-equivalent of the nitrogen lost, computing this nitrogen to urea or ammonium carbonate.

[4] In experiment 221a the feces and urine were collected together, in the calorimeter, while during the other days of the digestion period they were collected separately.

TABLE III.—*Loss of nitrogen and carbon during the drying of cow's feces-and-urine mixture*

Experiment and period	Dry matter per day	Nitrogen lost per day	Carbon lost per day		
			Total	Combined with nitrogen (computed from N lost)	Not combined with nitrogen
221f:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
874 I	2,225.8	78.3	71.9	33.6	38.3
874 II	1,516.0	50.3	58.8	21.6	37.2
887 I	1,887.4	82.0	70.7	35.2	35.5
887 II	1,296.5	59.2	48.5	25.4	23.1
886 I	3,178.8	83.9	119.6	36.0	83.6
886 II	2,739.8	96.8	124.7	41.5	83.2
221g:					
887 I	2,793.1	57.7	108.3	32.5	75.8
887 II	1,405.3	58.5	82.2	25.1	57.1
887 III	2,072.2	61.4	79.3	26.3	53.0
887 IV	2,924.1	87.3	83.5	37.5	46.0

To diminish this possibility of error in the carbon balance, in those cow experiments in which the carbon was not determined in the fresh feces-and-urine mixture, the loss of carbon on drying, per gram of nitrogen lost, was computed from the average of the data available, this average being 1.15 gm. and the correction for carbon lost on drying was computed by the use of this factor. That this correction is not entirely satisfactory is realized, since the loss of carbon does not in all cases follow exactly the loss of nitrogen, but it was considered, under the conditions, as the best possible way of correcting these earlier results which were obtained from the air-dried material.

LOSS OF ENERGY IN AIR-DRYING FECES-AND-URINE MIXTURE

A loss of nitrogen and carbon on drying does not mean a loss of only these elements, but it also represents a certain loss of potential energy. While the original fresh feces-and-urine mixture might have contained some carbon as free CO_2, the quantity so contained, in view of its solubility, could be only a very small fraction of the total quantity lost during the drying. Some of this loss no doubt came from the decomposition of urea, and this portion has been estimated in the experiments from the nitrogen lost. The remainder has been assumed, in the absence of definite knowledge, to be a result of carbohydrate fermentation, since the conditions of the drying are regarded as favorable for such a process. On this basis the energy lost on drying has been computed by using Rubner's factor, 5.45 for loss in Calories per gram of nitrogen lost, and the factor 9.4 for computing Calories per gram of carbon lost in excess of that required to satisfy the nitrogen lost, the latter factor representing the energy per gram of carbon in starch.

Although the above computation is not without logical justification, one must admit the possibility of considerable error in this correction, in view of our imperfect knowledge as to the nature and extent of the losses. Since it is impracticable directly to determine the energy in the fresh feces-and-urine mixture, the drying process may be, therefore, a source of serious error in the computation of the heat production.

To illustrate the extent to which the above considerations, as to losses and corrections may affect the computed heat production Table IV has been compiled, the data in the three methods of computation being derived from the data of experiments 221f and 221g. In all three cases the nitrogen was determined in the fresh substance, and energy in air-dry substance. In Method I carbon was determined in the air-dry material. Corrections for carbon and energy were computed from the nitrogen lost, calculated as urea. In Method II carbon was determined in the fresh material, corrections for energy being computed as in Method I. In Method III the carbon was determined in the fresh material and the corrections for energy were computed as in Method I, with an additional correction for carbon lost in excess of that required to combine with the nitrogen to form urea (using the factors 5.45 per gram nitrogen and 9.4 per gram carbon).

TABLE IV.—*Computed heat production as affected by loss of carbon during drying*

Experiment No.	Cow No.	Period No.	Method I. Carbon of air-dry substance; carbon and energy corrected for loss of N		Method II. Carbon of the fresh substance; energy corrected for loss of N		Method III. Carbon of the fresh substance; energy corrected for loss of N and for C uncombined with N	
			Computed heat production	Computed ÷ observed	Computed heat production	Computed ÷ observed	Computed heat production	Computed ÷ observed
			Calories	*Per cent*	*Calories*	*Per cent*	*Calories*	*Per cent*
221f	874	I	11,163.7	101.8	11,639.2	106.2	11,279.2	102.9
		II	9,334.0	102.8	9,794.9	107.9	9,445.2	104.1
221f	887	I	10,542.4	102.1	10,983.2	106.4	10,649.5	103.1
		II	8,663.8	103.2	9,142.6	108.9	8,733.4	104.0
221f	886	I	12,923.8	95.9	13,972.1	102.9	13,176.0	97.0
		II	12,017.1	96.7	13,050.2	105.1	12,268.1	98.8
221g	887	I	12,472.7	100.8	13,413.8	108.5	12,701.3	102.7
		II	8,715.6	101.0	9,424.6	109.2	8,887.9	103.0
		III	10,270.0	104.0	10,928.1	110.7	10,429.9	105.7
		IV	11,871.8	101.7	12,442.9	106.6	12,010.5	102.9

In consideration of the loss of carbon on drying the feces-and-urine mixture, the results of Method I are seemingly paradoxical. Why, one may ask, by using a figure for carbon in the feces and urine, known to be much lower than the correct value, does the computed heat production agree closely with the observed, in fact, in most cases, even more closely than when computed by Method III? The answer lies in the details of the computation. Referring to the general formula for heat production, H=F−E−G, it is seen that H remains unchanged if G is increased and E is decreased simultaneously by the same amount. When, in computing the balance of carbon, the carbon lost through fermentation is ignored, the figure for carbon in the excreta is low, and the apparent gain of carbon and, therefore, of energy (G) is correspondingly high or the loss low. On the other hand, if one ignores that portion of energy of the feces and urine which was lost in drying as a result of the fermentation, the energy of the feces and urine (and therefore E) is low. These two factors, therefore, oppose each other, the net effect being the difference between the two. For example, in experiment 221f, 874–I, the carbon calculated to have been lost from the excreta by fermentation was 38.3 gm. This being ignored, the balance of carbon showed a gain which was 38.3 gm. too high. This is equivalent to 50.06 gm. of fat (38.3×1.307), or in terms of energy, 475.5 Calories (50.06×9.5). G is therefore 475.5 Calories too high. The heat lost in fermentation, computed from the carbon, was 360.0 Calories. This being ignored, E is too low by 360.0 Calories. The net effect on the computed heat production is therefore 360.0−475.5= −115.5 Calories, which accounts for the difference between I and III in Table IV (11,279.2−11,163.7=115.5).

To state this somewhat differently, since the metabolizable energy equals the gross energy of the feed minus the energy of the feces, urine, and methane, when the metabolizable energy and the energy of the body gain are increased simultaneously the net effect on the computed heat production equals the increase of the former minus the increase of the latter. Thus, in the case just cited, the metabolizable energy is too great by 360 Calories, while the energy of the body gain is too great by 475.5 Calories. The net effect on the computed heat production is, therefore, 360.0−475.5= −115.5 Calories.

Thus it is possible to obtain a figure for heat production, which may agree closely with the observed, as a result of a balance of opposing errors.

The results of Method II show unusually large differences between the computed and the observed heat production, the carbon in the feces and urine as determined in the fresh substance being used, and the energy lost in fermentation ignored.

The results of Method III are as they appear also in Table II. The basis for the computation and the possibilities of error have already been considered.

The results as set forth in Tables III and IV indicate the need of rigorous control of the conditions of drying the feces and urine, and further investigation of the nature of the material lost during drying.

Recurring now to the computation of the heat production in the steer experiments, the feces and urine were collected, dried, and analyzed separately. The carbon in the urine was determined in the fresh sample, and the correction for energy lost on drying was computed from the nitrogen lost. The carbon in the feces was, however, determined in the air-dried sample, ignoring the possibility of loss of carbon and energy during the drying. There is no data covering the losses on drying steer feces. There is, however, data which show a considerable loss of carbon in drying cow feces (not a mixture of urine and feces), which also implies a loss of energy. The results of the analysis of several samples of cow feces, which were collected by an attendant on a single day of the digestion experiments, are given in Table V.

POSSIBLE ERROR IN THE DETERMINATION OF DRY MATTER OF FECES-AND-URINE MIXTURE

The determination of the dry matter of the feces-and-urine mixture is another source of error to which attention is called.

For determinations of energy, nitrogen, carbon, etc., in the combined feces and urine, the composite sample representing several days' collection was used. In all cow experiments this composite was made up of 10 daily aliquots. The dry matter, however, was determined in this composite and also in each of the daily samples. The same routine was followed in the drying of the daily samples as in the case of the composite, except that the former were generally left in the drying closet for a longer period of time, since, as a rule, they were placed in the closet as soon as obtained, and were allowed to remain until the composite sample also was thoroughly dry.

TABLE V.—*Losses of nitrogen and carbon occurring during the drying of cow feces*

Experiment and period	Dry matter of feces	Loss of nitrogen		Loss of carbon		Loss of carbon per gram nitrogen lost
	Grams per day	*Per cent* •	*Grams per day*	*Per cent* •	*Grams per day*	*Grams*
221f:						
874 I	2,013.7	0.092	1.86	1.045	21.0	11.29
874 II	1,409.7			1.208	17.0	
887 I	1,560.2	.367	5.73	3.379	52.7	9.20
887 II	1,086.3	.240	2.61	1.891	20.6	7.89
886 I	2,762.2	.236	6.53	1.872	51.7	7.92
886 II	2,796.0	.208	11.41	3.114	87.1	7.63
221g:						
887 I	2,755.4	.165	4.54	1.672	46.1	10.15
887 II	1,147.2	.124	1.43	1.000	11.5	8.04
887 III	1,583.4	.160	2.53	1.384	21.9	8.66
887 IV	2,665.1	.096	2.56	3.228	86.3	33.71

• Computed to dry-matter basis.

These results show that while the loss of nitrogen is slight, the loss of carbon is several times as great.

It would, of course, be unsafe to assume that identical losses occurred in the drying of steer feces since the composition of the rations, and, therefore, presumably, of the feces differed from that applying to the experiments with cows. Furthermore, the losses on drying are shown to be too variable to be regarded as accurately applylng to other conditions. However, the data suggest the probability of significant loss of carbon during the drying of steer feces also.

The bearing of the facts as to losses on drying, as disclosed by the above data, is also obviously important in connection with digestion experiments.

In all cases but one the percentage of dry matter as determined on the composite was found to be higher than the true average of determinations on the daily samples. Such a uniform difference must have an explanation other than errors of sampling or weighing. Aside from these errors, such differences could be due either to an unweighed evaporation of moisture from the composite during preparation, or to a greater loss of dry matter during the drying of the daily samples. Considering the precautions taken to prevent evaporation the loss by this means could have been but slight. The loss of dry matter due to fermentation

during the drying of the daily samples appears, therefore, to be the more extensive factor of error, but since there must have been some error on both these accounts the true figure for dry matter must have been higher than the one and lower than the other estimation, and there being no means of determining this intermediate figure exactly, the average of the two was used.

Table VI gives the results of the determinations of dry matter in the composite sample, the average of determinations in the daily samples, and the average of the two, as well as the possible error, in per cent and in grams of dry matter per day.

The differences between the two percentages of dry matter are fairly uniform. The average of all differences (omitting that of experiment 221c, 886 I) is 0.869 per cent. This means that, on the average, the dry matter as determined on the composite is about 0.9 per cent higher than the true average of the daily determinations or that the possible error of using the average of the two is about ±0.4 per cent. The magnitude of the error in grams per day obviously depends on the fresh weight of the material, and ranges in these experiments from ±14.4 gm. to ±113.2. It should be understood that the data presented apply only to the particular conditions and procedures which prevailed in this work.

An error in the determination of dry matter involves corresponding errors in energy, nitrogen, and carbon as determined on the dried material, all being in the same direction. These possible errors have been computed in Table VII, using the data from experiments 221f and 221g as examples. The possible errors in energy range from ±135.4 Calories to ±451.9 calories; the possible errors in nitrogen are small, ranging from ±0.59 gm. to ±2.46 gm.; the possible errors in carbon range from ±13.49 gm. to ±44.96 gm.

TABLE VI.—*Possible error of determinations of dry matter in feces-and-urine mixture*

Experiment and period	Fresh weight	Dry matter			Possible error	
		I Composite sample	II True average of daily samples	III Average of I and II		
	Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Grams dry matter*
221c:						
885 I	20,038.4	13.182	12.312	12.747	±0.435	±87.2
885 II	14,044.4	13.170	11.946	12.558	±.612	±86.0
885 III	14,785.4	15.050	13.810	14.430	±.620	±91.7
886 I	17,137.8	12.595	15.097	13.846	∓1.251	∓214.4
886 II	12,121.0	13.044	12.242	12.643	±.401	±48.6
886 III	17,364.2	15.973	14.669	15.321	±.652	±113.2
221d:						
885 I	9,415.2	14.740	13.848	14.294	±.446	±42.0
885 II	16,614.8	12.268	11.520	11.894	±.374	±62.1
885 III	10,410.8	13.391	12.555	12.973	±.418	±43.5
886 I	9,258.9	15.511	14.523	15.017	±.494	±45.7
886 II	15,567.8	14.429	13.715	14.072	±.357	±55.6
886 III	10,464.6	13.178	12.468	12.823	±.355	±37.1
221e:						
885 I	11,784.9		14.098			
885 II	8,937.8	13.954	13.094	13.524	±.430	±38.4
886 I	17,992.2	15.942	14.956	15.449	±.493	±88.7
886 II	15,846.1	15.681	15.007	15.344	±.337	±53.4
874 I	14,513.1	17.373	16.263	16.818	±.555	±80.6
874 II	11,900.2	17.099	16.857	16.978	±.121	±14.4
221f:						
874 I	13,370.7	17.131	16.163	16.647	±.484	±64.7
874 II	9,018.0	17.362	16.206	16.784	±.578	±52.1
887 I	13,424.0	14.514	13.588	14.051	±.463	±62.2
887 II	8,909.0	14.992	14.110	14.551	±.441	±39.3
886 I	20,344.0	16.073	15.099	15.586	±.487	±99.1
886 II	18,668.0	14.830	14.510	14.670	±.160	±29.9
221g:						
887 I	16,924.0	16.903	15.965	16.434	±.469	±79.4
887 II	10,220.0	14.178	13.322	13.750	±.428	±43.7
887 III	14,022.0	15.126	14.416	14.771	±.355	±49.8
887 IV	18,531.0	16.102	15.442	15.772	±.330	±61.2

TABLE VII.—*Effect of possible error in the dry matter of feces-and-urine mixture on the energy, nitrogen, carbon, and computed heat production*

Experiment and period	Possible error in daily dry matter (from Table VI)	Energy per gram dry matter	Carbon in dry matter	Nitrogen in dry matter	Possible error in energy	Possible error in carbon	Possible error in nitrogen	Energy equivalent to the carbon C×9.4	Energy equivalent to the nitrogen N×5.45	Energy equivalent to both N and C	Possible error in computed heat production
221f:	*Grams*	*Calories*	*Per cent*	*Per cent*	*Calories*	*Grams*	*Grams*	*Calories*	*Calories*	*Calories*	*Calories*
874 I....	±64.7	4.425	43.801	2.845	±286.3	±28.34	±1.84	±266.4	±10.0	±276.4	∓9.9
874 II...	±52.1	4.381	43.648	2.975	±228.3	±22.74	±1.55	±213.8	±8.4	±222.2	∓6.1
887 I....	±62.2	4.451	44.207	2.397	±276.9	±27.50	±1.49	±258.5	±8.1	±266.6	∓10.3
887 II...	±39.3	4.394	43.890	2.590	±172.7	±17.25	±1.02	±162.2	±5.6	±167.8	∓4.9
886 I....	±99.1	4.560	45.368	2.485	±451.9	±44.96	±2.46	±422.6	±13.4	±436.0	∓15.9
886 II...	±29.9	4.528	45.101	1.980	±135.4	±13.49	±0.59	±127.3	±3.2	±130.5	∓4.9
221g:											
887 I....	±79.4	4.555	45.469	2.557	±361.7	±36.10	±2.04	±339.3	±11.1	±350.4	∓11.3
887 II...	±43.7	4.547	45.303	2.464	±198.7	±19.80	±1.08	±186.1	±5.9	±192.0	∓6.7
887 III..	±49.8	4.493	45.123	2.360	±223.8	±22.47	±1.18	±211.2	±6.4	±217.6	∓6.2
887 IV..	±61.2	4.545	45.323	2.258	±278.2	±27.74	±1.38	±260.8	±7.5	±268.3	∓9.9

It will be noted that the possible errors in energy of the feces-and-urine mixture, as a result of the possible errors in dry matter, appear to be appreciable, and they would remain so if the nitrogen and, especially, the carbon as determined on the dried substance were not used as a basis for computing the loss of nitrogen and carbon on drying and of an energy correction corresponding to these losses.

The differences between the nitrogen and carbon as determined on the dry substance and those determined on the fresh represent the loss on drying. A positive error in the carbon and nitrogen determined on the dried substance would, therefore, mean a negative error in the computed loss on drying and, consequently, a negative error in the computed energy correction when such a correction is applied, and vice versa. As a result of this, the initial error in energy is reduced by the energy equivalent of the error in the computed loss of nitrogen and carbon during drying. These energy equivalents and the net effect on the computed heat production are shown in Table VII. The resultant error is negligible according to this method of computation. For example, if the error in dry matter in experiment No. 221f, 874I were +64.7 gm. (see Table VII), the energy of the daily feces-and-urine mixture, as computed by multiplying grams dry matter by the number of Calories per gram, would be too great by the amounts indicated in column 5, namely 286.3 Calories (64.7 times 4.425). Similarly, the figures for carbon and nitrogen as determined on the dry substance would be too high by the amounts indicated in columns 6 and 7 respectively (64.7 times $\frac{43.801}{100}$=28.34 gm. carbon and 64.7 times $\frac{2.845}{100}$=1.84 gm. nitrogen). On the other hand, by subtracting the too large amounts of carbon and nitrogen in the dry material from those determined on the fresh the results for loss of carbon and nitrogen on drying, thus obtained, would be too small by the same amounts given in columns 6 and 7. The energy corrections based on these losses would then be too small by the amounts indicated in columns 8 and 9, totaling in the case cited 276.4 Calories (column 10), and making the final error in the energy of these excreta equal 286.3−276.4=9.9 Calories, or in the computed heat production, −9.9 Calories.

DETERMINATION OF DRY MATTER IN FEED

It is obvious that an error in the determination of the dry matter of the feed would directly affect its energy equivalent, and also the carbon and nitrogen balances.

Dry matter is determined by first subjecting the sample to a preliminary determination of the air-dry substance; the sample is then ground, and the remaining hygroscopic moisture is determined in the ground sample. If there is any loss of moisture during the grinding, this is not accounted for in the determination, and would increase the figure for dry matter in the feed.

The effect of grinding on the dry matter determination is a very variable factor, the exact magnitude of which is unknown, but a preliminary investigation of this problem indicates that the

grinding of the samples may be a source of appreciable error in the dry matter determination. However, the effect of an error in the dry matter of the feed, on the computed heat production, would be relatively slight, for the reason that the metabolizable energy and the energy of the body gain would be affected in the same direction. This is illustrated by the following computation, the basis being arbitrarily assumed figures, and the effect on the nitrogen balance being disregarded:

Daily ration	8,000 gm. air-dry matter.
Error in dry matter	+0.5 per cent.
Energy per gram dry matter	4.5 Calories.
Carbon in dry matter	45 per cent.
Error in the daily dry matter	+40 gm.
Error in the metabolizable energy	40×4.5=+180 Calories.
Error in carbon balance	+18 gm.
Error in energy of the body gain	18×1.307×9.5=+223 Calories.
Error in computed heat production	180−223=−43 Calories.

OTHER SOURCES OF ERROR

THE GLYCOGEN CONTENT OF THE BODY.—The computation of the energy equivalent of the gain by the animal assumes that the glycogen content of the animal remains unchanged. With a submaintenance ration, or in the case of milking cows, when the ration is not sufficient fully to support the milk production, the possibility of a continuous loss of glycogen for some time may be considered. In the event of such loss the estimate for the energy equivalent of the body loss may be too large. In those cases in which there is a gain of body substance, after a considerable number of days of preliminary feeding on the ration of the experimental period to follow, the possibility that the glycogen content of the animal will come into equilibrium with the ration is greater, and the error involved in the assumption of a constant glycogen content of the body may not be appreciable.

CARBON EQUIVALENT TO THE NITROGEN LOST BY THE BODY.—Whether nitrogen be gained or lost by the body, it is considered to be accompanied by such an amount of carbon as is contained in an equivalent amount of protein, carbon being considered as constituting 52.54 per cent of the protein. The gain or loss in fat is computed from the carbon balance only after the carbon content of the protein gained or lost has been set aside. In case of a loss of protein, if there is a utilization of a part of the nonnitrogenous fraction of the katabolizing tissue protein the amount so utilized is not accounted for, and constitutes an error in the computation of the carbon equivalent to the nitrogen lost and, likewise, in the carbon balance.

CARBON DIOXIDE OF THE WATER CONSUMED.—The carbon dioxide content of the water consumed by the animal is usually not taken into consideration. This introduces a small error into the carbon balance, tending to reduce the apparent gain of carbon and to make the computed heat production too great.

HEAT OF HYDRATION AND SOLUTION.—Another small error is introduced in the determination of the energy of the visible excreta by neglecting the heat of hydration and solution. If heat is absorbed when solids come in contact with water, the reverse must be true, that is, heat is evolved when water is driven off. Conversely, if heat is evolved when solids are brought in contact with water, this heat must be absorbed on driving off the water. Thus, it is known that when urea is brought into solution an absorption of heat takes place, while when proteins are brought into contact with water an evolution of heat takes place. The feed is ordinarily given to the animal in an air-dry condition. Hydration and solution take place in the animal body, and corresponding evolution and absorption of heat must result. These processes may be considered as factors in the work of digestion. The energy of the urine, feces, and milk is determined after these substances have been dried. The heat of hydration and solution is not accounted for in the determination.

SUMMARY OF OBSERVATIONS ON SOURCES OF ERROR IN THE INDIRECT METHOD

Errors on the following accounts are either unavoidable or are of negligible magnitude: Possible change in the glycogen content of the body of the animal; possible utilization of the nonnitrogenous portion of the katabolized protein molecule; and the energy of hydration and solution.

It seems possible that errors due to the two factors first mentioned might be of appreciable importance in experiments in which the subjects are under submaintenance conditions.

A small determinable error is introduced by the carbon dioxide of the drinking water.

By far the most serious possibilities of error seem to lie in the methods of preparing the samples of feed, feces,

and urine for analysis. Of these the drying of the feces-and-urine mixture is the most important, since it involves the computation of corrections, the bases for which are imperfectly understood.

DIRECT HEAT MEASUREMENT

The direct measurement of the heat given off by the animal body is accomplished by means of the respiration calorimeter. Descriptions of such an apparatus, as used at this institute, and also of the methods employed, have appeared in several publications by Armsby and Fries (*3; 4, p. 263–270; 1; 5, p. 200–222*). For the present purpose the more important details may be condensed into the following:

The respiration calorimeter used at the Institute of Animal Nurtition of the Pennsylvania State College is of the Atwater-Rosa type, consisting of a Pettenkofer respiration apparatus, the chamber of which is also an animal calorimeter.

The respiration chamber is built with a double metallic wall inclosing a 3-inch dead-air space, surrounded, at a distance of 4 inches, by a wooden wall, and this in turn, at a distance of 4 inches by a second wooden wall, thus forming two air-spaces surrounding the chamber. By the heating or cooling of these air spaces the inner wall is maintained adiabatic.

The sensible heat given off by the animal, which constitutes the greater part (about three-fourths) of the total heat production, is absorbed by a current of cold water passing through copper pipes at the top of the respiration chamber, the exposure of these pipes to the air within the chamber being regulated by means of shields which can be raised or lowered by the operator. By this means the rate of removal of heat from the chamber is adjusted as required for the maintenance of an approximately constant temperature.

The temperature of the in-going air is maintained equal to that of the outcoming.

The temperature of the in-going and outcoming water is read every four minutes by means of two mercurial thermometers, graduated to 1/50° C., and carefully calibrated.

The volume of water passing through the calorimeter is measured by means of two copper meters of 100-liter capacity.

For each period of uniform water flow the product of the amount of water passing through the heat absorbers multiplied by the average temperature difference equals the amount of heat removed from the chamber in the water current.

Since a part (approximately one-fourth) of the total heat given off by the animal is in the form of latent heat of water vapor, this portion of heat, not being directly measurable by the calorimeter, is calculated from the amount of water vapor given off, using 0.587 Calorie per gram at 18° C. This heat is added to the heat removed in the water current to get the total heat emission.

CORRECTIONS

In order accurately to determine the amount of heat produced by the animal several corrections are made, for possible errors in the readings, for heat measured by the apparatus but not coming from the animal, and for heat withdrawn from the chamber but escaping measurement. The following is the list of corrections usually made:

(*a*) Corrections for difference of pressure on the bulbs of the two thermometers.

(*b*) Correction for friction of water in absorbers.

(*c*) Correction for lag in the rise or fall in temperature of the water from that at the inlet to that at the outlet of the absorber system.

(*d*) Correction for change in temperature of the absorber system during the experiment.

(*e*) Correction for heat developed by the blades of the fan used to stir the air in the calorimeter.

(*f*) Correction for change in temperature of the walls of the chamber during the experiment.

(*g*) Corrections for heat introduced into the apparatus or withdrawn from it, in feed, drink, excreta, and vessels containing these materials.

(*h*) Correction for the metabolism of the man entering the chamber during the experiment, to milk the cow.

(*i*) Correction for condensation of water on the absorber system.

(*j*) Correction for storage or loss of heat due to gain or loss of matter by the animal body during the experiment.

ACCURACY OF THE DIRECT METHOD

This list of the corrections serves to indicate the degree of accuracy which it is sought to attain. There are, however, two other sources of error which have been disregarded

because no practicable methods of measuring or estimating corrections have been found.

These errors depend on changes of body temperature of the animal during an experiment, and change in amount of moisture upon the walls of the chamber during an experiment.

Assuming, under the conditions of the experiments, the possibility of a change of ±0.6° F. (*8, p. 24-26*) (0.3° F. per day, in the same direction during a two-day period), the possible error in the daily heat determination, in the case of an animal weighing 400 kg., would be ±53 Calories.

As to the mosture factor, there is the possibility of a deposit of moisture on the walls of the chamber, or of an evaporation of moisture from the same, during the experiment, thus constituting an error in the measurement of the latent heat of water vapor produced. Under ordinary conditions this error is probably not appreciable. Its magnitude and direction would depend on the initial humidity in the chamber, length of the preliminary period, rate of ventilation, and water vapor given off by the animal. It is apparent that this error would be greater in short-time experiments than in those of longer duration.

As regards the ultimate accuracy of the direct method, from a consideration of the results of the 18 alcohol check tests reported from this institute by Armsby and Fries (*5, p. 200–222*) the authors came to the conclusion that the results of a single experiment with the respiration calorimeter may be regarded as accurate to within approximately the following percentages of the amounts determined: Heat, 1 per cent; water, 6.0 per cent.

The determination of the carbon dioxide production, which is used in the indirect method, is regarded as accurate to within approximately 0.5 per cent of the amount determined.

The results of 14 alcohol checks made subsequent to the above-mentioned report, in practically the same manner as before, and using heat values for anhydrous alcohol ranging from 7.07537 to 7.13107 Calories per gram, show also a close agreement between the computed and the observed values, the average percentages recovered being as follows: Heat 98.9 per cent; water, 108.0 per cent; carbon dioxide, 99.9 per cent. The results of these 14 individual tests are set forth in Table VIII.

It will be noted that in all these tests the observed heat is less than the computed. This rather uniform difference may be due either to the assignment of too high a heat value to the alcohol, or to the heat measurement, or to both. The range of the differences, however, is narrow. Considering the results of all the alcohol checks, and the fact that the possibility of error in short-time experiments is greater than in experiments of longer duration, the conclusion stated above, with regard to the accuracy of the direct heat measurement as well as to the carbon dioxide determination, seems to be warranted.

TABLE VIII.—*Results of alcohol check tests*

Date	Number of hours	Heat			Carbon dioxide			Water		
		Observed	Computed	Observed ÷ computed	Observed average of aspirator and meter	Computed	Observed ÷ computed	Observed average of aspirator and meter	Computed	Observed ÷ computed
		Calories	*Calories*	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Per cent*
Nov. 23, 1911	8	4,085.4	4,144.7	98.6	1,097.0	1,110.8	98.8	732.6	739.3	99.0
Dec. 31, 1912	8	4,252.6	4,302.0	98.9	1,170.1	1,152.9	101.4	852.2	770.0	110.7
Apr. 29, 1913	7	3,778.9	3,817.2	99.0	1,022.4	1,023.0	99.9	759.9	683.2	111.2
Dec. 4, 1913	7	3,426.8	3,491.4	98.2	928.8	935.7	99.3	679.3	631.9	107.5
Apr. 28, 1914	7	3,397.9	3,440.9	98.8	921.1	922.1	99.9	698.9	622.8	112.2
Dec. 17, 1914	7	3,481.5	3,507.7	99.3	939.5	940.1	99.9	671.8	634.9	105.8
June 23, 1915	7	3,462.8	3,478.3	99.6	931.1	932.2	99.9	689.4	629.3	109.6
May 9, 1916	7	3,507.9	3,584.5	97.9	965.1	960.6	100.5	673.0	647.4	104.0
Dec. 14, 1917	7	3,294.8	3,331.0	98.9	897.2	898.3	99.9	651.6	606.4	107.5
Jan. 3, 1918	7	3,252.1	3,310.5	98.2	884.4	893.5	99.0	637.1	602.3	105.8
Apr. 20, 1920	8	3,534.0	3,540.8	99.8	• 955.8	956.4	99.9	• 744.9	644.6	115.6
Apr. 21, 1921	8	3,383.4	3,390.7	99.8	• 908.4	915.9	99.2	• 672.1	617.2	108.9
Apr. 20, 1922	8	3,429.6	3,461.1	99.1	945.0	934.9	101.1	• 676.0	630.1	107.3
Nov. 24, 1922	8	3,495.2	3,542.3	98.7	949.4	949.6	100.0	685.7	637.7	107.5
Total		49,782.9	50,343.1		13,515.3	13,526.0		9,824.5	9,097.1	
Average		3,555.9	3,595.9	98.9	965.4	966.1	99.9	701.8	649.9	108.0

• By aspirator only.

SUMMARY OF DIRECT AND INDIRECT HEAT ESTIMATION

The results of the study of the details of direct and indirect calorimetry in experiments with steers and cows at this institute may be summarized as follows:

The direct heat measurement is in general more accurate than the balance method of indirect heat computation. The direct method, therefore, serves as a valuable check on the accuracy of the analytical work involved in the indirect method.

In the measurement of the nutritive values of feeds, however, the direct and the indirect methods are about equally accurate, and the latter is much the more easily accomplished.

One source of error, not affecting direct heat measurement, but involved in the utilization of both direct and indirect heat measurements in the determination of the net energy values of feeds, is the loss of moisture of feeds and feces during grinding preparatory to analysis, but this factor affects both methods.

The most important source of error affecting direct and indirect calorimetry in different ways is loss of matter, from the urine and feces, presumably through fermentation, during drying, preliminary to the determination of energy by means of the bomb calorimeter.

This loss, which contains carbon and nitrogen, is an important factor in the computation of the heat production by the indirect method, and in the application of the results of the direct method in determining the net energy value of feeds.

This loss of matter affects the estimation of (*a*) metabolizable energy, as determined by subtracting from the energy of the feed the energy of the excreta, (*b*) heat production, as determined by subtracting from the metabolizable energy the energy of the gain (carbon and nitrogen balances multiplied by factors), (*c*) energy of gain, as determined by subtracting from the metabolizable energy the directly observed heat production.

The determination of the energy of the gain from the nitrogen and carbon balances does not involve the loss under discussion, since the nitrogen and the carbon can be determined on the fresh substance. When, however, the direct heat estimation is used in the final computation of net energy any such advantage as might accrue from the greater accuracy of this direct heat estimation is lost through the fact that in order to derive the amount of the energy of the gain, which is a factor of the net energy estimation, the directly observed heat production must be subtracted from the metabolizable energy, this last datum being directly affected by the loss in drying of the excreta.

In those cases in which there is a gain of body tissues, or no appreciable loss, the error due to possible change in glycogen content of the body may be considered as negligible, and the estimation of the gain in energy, by the indirect method, may be as accurate as when determined by the direct method.

On account of the possibilities of change in the glycogen content of the bodies of cows, in connection with milk production, the chances for error in indirect calorimetry with cows seem greater than with steers.

There is need for careful control of the conditions of drying feces and urine, and of a thorough investigation into the composition and nature of the substances lost, in order to make possible the more accurate estimation of the dry matter and the energy of these excreta.

LITERATURE CITED

(1) ARMSBY, H. P., and FRIES, J. A.
1903. THE AVAILABLE ENERGY OF TIMOTHY HAY. INVESTIGATIONS WITH THE RESPIRATION CALORIMETER. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 51, 77 p., illus.

(2) ———
1903. THE PRINCIPLES OF ANIMAL NUTRITION. WITH SPECIAL REFERENCE TO THE NUTRITION OF FARM ANIMALS. 614 p., illus. New York and London.

(3) ———
1904. THE RESPIRATION CALORIMETER AT THE PENNSYLVANIA EXPERIMENT STATION. Exp. Sta. Rec. 15: 1037–1050, illus.

(4) ———
1908. INVESTIGATIONS IN ANIMAL NUTRITION. U. S. Dept. Agr., Bur. Anim. Ind. Ann. Rpt. (1906) 23: 263–285, illus.

(5) ——— and FRIES, J. A.
1911. THE INFLUENCE OF TYPE AND OF AGE UPON THE UTILIZATION OF FEED BY CATTLE. U. S. Dept. Agr., Bur. Anim. Ind. Bul. 128, 245 p., illus.

(6) ———
1913. A COMPARISON OF THE OBSERVED AND COMPUTED HEAT PRODUCTION OF CATTLE. Jour. Amer. Chem. Soc. 35: 1794–1800.

(7) KELLNER, O., and KÖHLER, A.
1900. UNTERSUCHUNGEN ÜBER DEN STOFF- UND ENERGIE-UMSATZ DES ERWACHSENEN RINDES BEI ERHALTUNGS- UND PRODUKTIONSFUTTER. Landw. Vers. Stat. 53: 1–474.

(8) KRISS, M.
1921. OBSERVATIONS ON THE BODY TEMPERATURE OF DRY COWS. Jour. Agr. Research 21: 1–28, illus.

(9) LAVOISIER, [A. L.], and LAPLACE, [P. S.]
1784. MÉMOIRE SUR LA CHALEUR. Hist. Acad. Roy. Sci. [Paris] 1780: 355–408, illus.

(10) RUBNER, M.
1894. DIE QUELLE DER THIERISCHEN WÄRME. Ztschr. Biol. (n.F. 12) 30: 73–142, illus.

THE LIFE HISTORY OF PILACRE FAGINEA (FR.) B. & BR.[1]

By C. L. SHEAR, *Senior Pathologist*, and B. O. DODGE, *Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Pilacre is a fungus holding an important place in mycology, primarily on account of Brefeld's contribution to its life history and relationship. We may not agree with him as to the lines of evolution which he lays down, nor be able to confirm all his work as to questions of fact in each case. No one, however, has given a finer illustration of the kind and quality of work to be done in support of a theory than did Brefeld in his study of "*Pilacre petersii* B. & C." The writers have followed the life history of this "primitive basidiomycete" in pure cultures. In addition to confirming in the main Brefeld's work, as far as he was able to carry his investigation, the writers here give additional information which should be helpful in clearing up the question of the relationship of *Pilacre faginea* (Fr.) B. & Br. to the great groups of fungi.

Basing his argument on the old doctrine that, so far as structures in fruiting organs are concerned, evolution proceeds from the indefinite to the definite, and from larger numbers of organs to smaller numbers, Brefeld strongly supported the view that the basidium with its four spores has developed as a reduction process from a septate conidiophore which originally gave rise to an indefinite number of conidia. One of his lines of evolution runs from the smuts, with their "hemibasidia" and yeastlike budding of spores, through the rusts, Auriculariaceae and Tremellaceae with their "protobasidia," each of the four cells producing a single spore, to the autobasidiomycetes of the mushroom type. It is in the line of "Protobasidiomycetes" that Pilacre is brought forward in support of the main argument in favor of his theory, namely, that basidia and conidiophores, and basidiospores and conidia are, respectively, homologous.

According to Brefeld, the small, grayish sporocarps of Pilacre frequently found on old beech logs are the equivalent of the ordinary mushroom fruit body. In dissecting the fruiting heads he found that the spores were formed on true basidia. Each basidium had exactly four cells and each cell bore only one spore. Though recognizing the very dissimilar characters of the fruiting structure, he places Pilacre among the "Protobasidiomycetes" next to the Auriculariae, but differing from the Auricularias in being angiocarpous and in having a gleba. The basidia were not developed in a definite hymenial layer.

In Brefeld's first cultures (*6*)[2] obtained by germinating spores from the fruiting heads only a hyphomycetous conidial form developed. In view of the fact that he could find no such conidial stage described and had not found it in nature he assumed that its production was due to the unnatural environmental conditions of his culture.

MATERIAL AND METHODS

Pilacre faginea has been collected by the senior author in localities as widely separated as New York and Florida. Although it occurs most frequently on beech logs it has been found on Acer, Carpinus, and other hardwoods, and typical specimens were found growing in the Florida everglades on wood of some subtropical tree.

The material for the cultures upon which the discussion in this paper is based was obtained in September, 1921, from specimens growing on a beech log near Chain Bridge, Va., a few miles from Washington.[3] Poured plates of corn-meal agar were made, using spores from the fruiting head. Sixteen single spore cultures were isolated and grown either at room temperature or in the refrigerator. Subcultures were grown on corn-meal

[1] Received for publication June 16, 1924—issued May, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 417.
[3] The writers are indebted to Angie M. Beckwith for assistance in the culture work.

agar, beech-decoction agar, and on sterilized beech branches. Large test tubes about 1 inch in diameter and 12 inches long proved to be most satisfactory for this work. As only one of the first cultures growing in small test tubes developed the basidial stage, other single-spore cultures were transferred to the large test tubes. These cultures were kept at room temperatures at first, but later some of them were placed in the refrigerator.

CHARACTERISTICS OF THE FUNGUS IN CULTURE

The basidiospores germinate in the manner described by Brefeld (*6*). The hyphae with their much branched conidiophores and luxuriant development of conidia have been so adequately and accurately described and figured by this author that further space need not be given to this phase of the question, except to call attention to certain characters not fully brought out by him. The mycelium in mass is not yellowish, as he describes. The production of conidia begins within a few days, and as soon as they cover the surface the color of the culture on potato-dextrose agar is Prout's brown, while on cornmeal agar it is Verona to sepia brown.[4] Grown on pieces of beech branches, the culture becomes covered with a great felty mass of mycelium and conidia, nearly Prout's brown in color. The mycelium grows very slowly; a colony several weeks old may not be over 2 cm. in diameter.

The fact that basidia, which are not easily recognized because of their form and manner of septation, are not developed in a hymenium and that their spores after becoming detached lie entangled among the loose hyphae of the head, has led some of our mycologists to a misconception as to the nature of the sporocarps. The hyphomycetous conidial stage which Brefeld first described and which always develops in cultures with the production of masses of conidia (pl. 2, A to C) comprises a phase in the life history entirely distinct from the basidial fructification.

After the first cultures had grown in 12-inch test tubes in the laboratory at room temperature for about four months, little flecks of pure white hyphae developed in several of the tubes. These growths increased in size, being at first rather indefinite in shape, but soon resembling very small mushroom buttons. The stipelike portion became surmounted by a small head. After two weeks some of the white growths began to turn grayish. These were removed and found to consist of hyphae having clamp connections. Basidia were developing from these hyphae in much the same way as figured by Brefeld. The basidiospores are nearly spherical, and being somewhat larger than the conidia they were easily distinguished from them. Brefeld has given adequate figures of the basidium and its spores. It is a true basidium having a definite number of spores, four in each case, just as claimed by him.

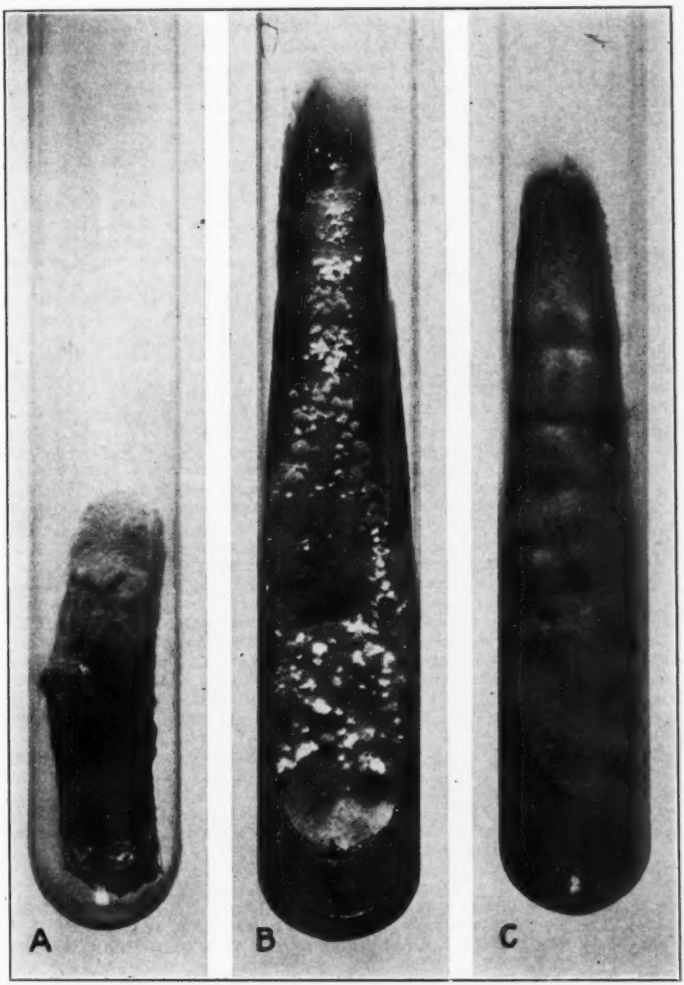
The first cultures, which were isolated November 5, 1923, fruited the following February, having meanwhile developed merely the gametophyte mycelium which produced only conidia. Sixteen monosporous cultures were transferred March 10; on June 5 it was found that most of the cultures showed the white patches of mycelium, which indicates that clamp connections are being formed and that basidia will soon develop. Ordinarily it requires about three months for the cultures to mature fruit bodies. One culture on potato-dextrose agar showed nearly 100 separate masses of white mycelium. Each mass represented an incipient fruit body covered with the ash-gray "gleba" in which quantities of basidiospores were being produced (pl. 1, B).

Cultures in which two monosporous strains in various combinations were grown did not produce the basidial fruiting stage in any greater abundance than did the single spore cultures. Fully as many fertile heads developed on cultures from one spore as were formed in cultures representing several strains.

No definite fruiting body such as characterizes Pilacre when it grows in nature on beech logs was developed in some of the cultures, although large numbers of basidia were formed in loose aggregations of hyphae. In other cases, however, fruiting bodies 2 or 3 mm. high were developed, some of them possessing a definite structure, consisting of a stipe surmounted by the expanded fertile head. The sporophytic hyphae of those which eventually produce basidia are pure white until the development of spores begins. The head then turns ash-gray as noted. Clamp connections are always found on the hyphae developing basidia. They also occur on the hyphae composing the so-called "peridium" (pl. 2, Q).

Wherever basidia are formed the hyphae producing them are clustered

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

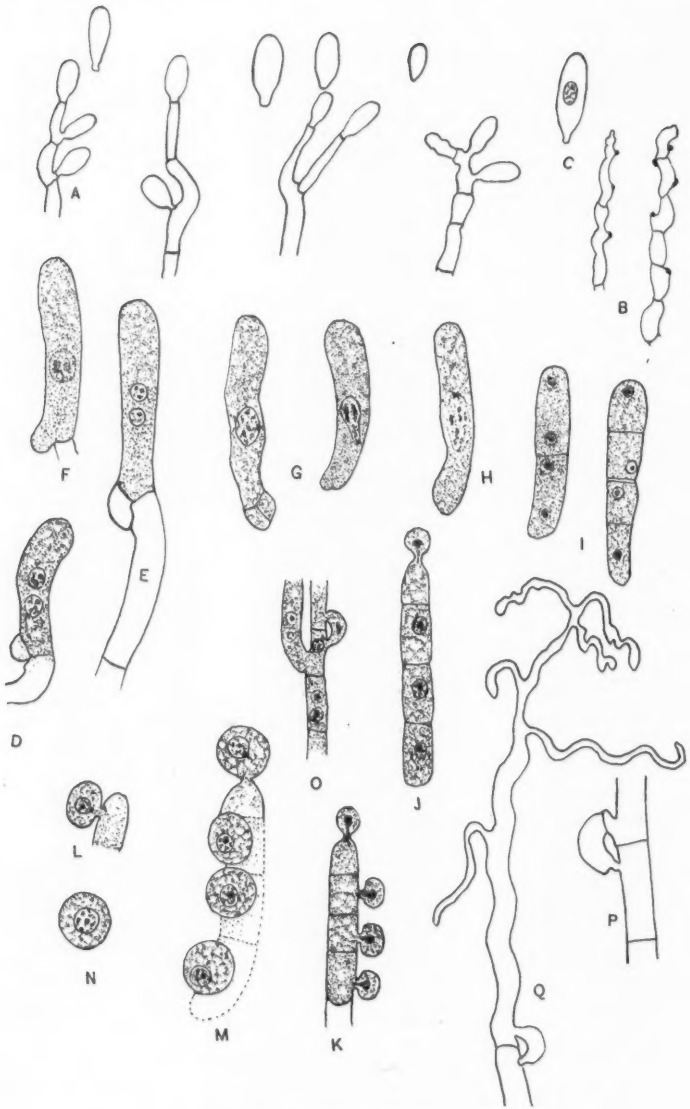


Cultures obtained by germinating basidiospores on potato dextrose agar, March 10, 1924. The cultures were photographed on June 9, natural size

A.—Single spore culture grown on sterilized beech twig showing only the conidial stage at the end of 3 months

B.—Parallel culture, also from a single spore; there was a large number of basidial fruit bodies developed on the surface of this culture

C.—Cultures which showed only the conidial stage after 3 months



The figures have the same magnification

A.—Ends of young conidiophores
B.—Rhinotrichum-like conidiophores
C.—Mature conidium
D to M.—Stages in the development of basidia and spores
N.—Mature spore
O to Q.—Cells of sporophytic hyphae showing clamp connections

together and not spread out indefinitely in the way one finds the mycelium producing basidia in Exobasidium or *Corticium vagum*, for example. A true peridium is not developed in Pilacre; the interlocking and entangling of the end branches of the sterile sporophytic hyphae make up an outer layer that has been called its peridium.

GAMETOPHYTIC AND SPOROPHYTIC GENERATIONS OF PILACRE

No fungus heretofore described illustrates more beautifully than does Pilacre the difference which may exist between the gametophytic and sporophytic stages in its life history. In this fungus the hyphae that produce conidia are gametophytic, those producing basidia are sporophytic. No conidia are developed on the latter kind of mycelium.

The gametophytic hyphae which arise from germinated conidia, basidiospores, or from bits of mycelium transferred to agar plates are of such a delicate structure and so narrow that it is difficult to differentiate cytoplasmic structures or to determine exactly the number of nuclei present in each cell. Some of the cells are extremely long and no doubt each cell then contains several nuclei. The conidia when first formed are uninucleated (pl. 2, C). Two masses of chromatin are sometimes visible in older spores, suggesting that the original nucleus may have divided. It is of interest to note that while no clamp connections can be found on the coarse brown hyphae-bearing conidia, these structures are easily found on the more delicate pure white hyphae which give rise to the basidia. Fixation of the sporophytic hyphae is rendered difficult by the quantity of air entangled in the hyphae of the "peridium." One occasionally finds cells with two nuclei (pl. 2, O). Sometimes cells are very long and these probably contain several nuclei. The fact that nuclei are sometimes found in pairs has no sexual significance. It is well known that when a fungus nucleus divides, the daughter nuclei tend to remain close together in pairs and only separate when they, in turn, divide. The branches that develop into basidia certainly arise at the clamp connection (pl. 2, D and E). The behavior of the nuclei at the time of clamp formation has not been followed, and an examination of additional material will be required in order to determine exactly what takes place at the origin of basidium. Presumably, the two nonsister nuclei which come together in a basidium clamp ordinarily fuse without further division. Brefeld (*6*) figures clusters of basidia in a way which suggests that there may be a great deal of proliferation at this time. It is well known that in the Ascomycetes a number of asci may arise as the result of such growth. The ultimate cell may also fuse with the antepenult, the resulting cell either becoming an ascus or by further growth and proliferation in turn give rise to another cluster of asci. Kniep's contention (*22*) that the clamps of Basidiomycetes and the crosiers of Ascomycetes are homologous structures would have been strengthened had he cited Brefeld's figures of Pilacre basidia as they arise from clamps. Such a suggestion implies that one accepts the theory that the Basidiomycetes and Ascomycetes have a common ancestral line. If so, there must be some very fundamental reason why the basidium becomes 4-nucleated and the ascus becomes 8-nucleated after the reduction divisions.

Such stages in the development of basidia as are shown in Plate 2, D to I, are abundant in sections of the sporocarps. It is clear that the cell wall in the middle of the basidium is laid down very early (pl. 2, I), even before the second division of the nucleus. The fusion nucleus, larger than either of the nuclei in the pair, is first seen. After a second division the nuclei are very small, each one containing a relatively large nucleolus. Figures obtained by the writers show that the basidium increases proportionately less in length than in diameter as it matures (pl. 2, J to M). It is not unusual to find one spore at the tip of the terminal cell (pl. 2, K). Not infrequently the sterigma is found at the side of the last cell of the basidium bringing all four spores into line (pl. 2, L). In Ascomycetes which have fruit bodies of any considerable size, by far the greater bulk of the tissue composing the ascocarp is gametophytic. The ascogenous hyphae proceeding from the ascogonium or from the cells which perform the function of an ascogonium are sporophytic in nature, and frequently compose in part what is called the subhymenium. In cleistocarpic ascocarps the tissue, sometimes erroneously called the peridium, which makes up the wall and overlies the hymenium, is gametophytic. The peridial hyphae of Pilacre are composed of the end branches of the hyphae, which are massed together below in parallel arrangement to form the stipe. Reaching the head, they develop the curiously contorted branches which, interlocking, form the peridium, through which the spores are slowly sifted out. As the fruit ages,

the peridial branches break away, exposing the spores tangled in the gleba. The peridial branches are first grayish in color and are scarcely to be distinguished from the rest of the young fruit body. As soon as spores are formed the head becomes ash-gray or cinereus. It is not difficult to determine by the change in color of the head when the first basidiospores are formed. As noted previously, the peridium is composed of sporophytic hyphae. This is proved by the fact that clamp connections regularly occur on these hyphae. In a culture bearing only conidia one looks in vain for such clamp connections, but as soon as the whitish flecks or mounds of mycelium develop on the conidial layer of the gametophytic mycelium, clamp connections can be found on those hyphae connected with the white mycelium above. The contrast between the coarse brown mycelium of the Rhizoctonia stage of *Corticium vagum* and the delicate whitish sporophytic hyphae bearing basidia is not greater than is found in the two stages in the life history of Pilacre.

Brefeld (*7*) drew his figures of hyphae bearing basidia and basidiospores from fruit bodies developing on bark. He germinated these spores and obtained cultures which produced only conidia. Later (*8, p. 164*) he reports producing the complete life cycle from basidiospores in culture. While these figures were drawn with the same care which he always gave to the details, when it comes to interpretation of his findings, he is moved to magnify similarities and to discount differences in comparing the basidia and their spores with the conidiophores and their conidia. As noted above, believing that a protobasidium is derived in the process of evolution from a conidiophore becoming septate or reduced to a definite structure with ultimately four cells, each cell producing a single spore, he says that the basidiospores and the conidia of Pilacre are about the same size, differ only a little in form, arise in exactly the same way, germinate exactly alike, and produce identical mycelia. In reality, the conidia and basidiospores are very different in color, they are absolutely dissimilar in form and are borne on hyphae with entirely different characteristics and origin. Of course the mycelium that develops from a germinated basidiospore could not differ from the mycelium which develops from a conidium, both structures being gametophytic. Hyphae giving rise to basidia are snow white, of delicate proportions, have clamp connections and sterile tip branches which take part in the formation of the "peridium." Clamp connections are now looked upon as an indication of the sporophytic condition of a mycelium. The recent work which has been done on the development of mushroom fruiting bodies from two strains of mycelium shows that in certain cases no clamp connections are formed in cultures from a single strain, but when two strains properly chosen are grown together so that anastomoses can occur, one begins to find clamp connections, and later the mushroom fruit body will develop. In such forms the binucleated condition of the hyphal cells is supposed to arise only along with or after clamp connections are developed.

The presence of clamp connections on hyphae is commonly accepted as an indication that the fungus belongs in the basidiomycete line, but failure to find such structures does not mean that the fungus must be excluded from this group. For example, Gilbert (*20*) did not find clamp connections on the hyphae of Dacryomyces. Here in some way, not determined, either as the result of anastomoses or by division of the nuclei, the cells below the hymenium become binucleated. After fusion of the two nuclei in the young basidium and the reduction divisions which follow, two of the four nuclei degenerate so that only two basidiospores are developed. Juel (*21*) as the result of cytological studies of *Stilbum vulgare* Tode believes that the fungus is a true Basidiomycete. He finds no clamp connections. On the other hand, the spores are regularly formed on club-shaped structures which originate as binucleated cells. The two nuclei then fuse. The fusion nucleus divides once and before the second division occurs a septum is laid down so that the basidium becomes 2-celled with one nucleus in each cell. The second division now occurs, but one of the daughter nuclei in each cell degenerates, so that only two basidiospores are developed. Juel points out that Pilacre and Pilacrella are angiocarpous while Stilbum is gymnocarpous. The peridium or covering surrounding the fertile tissue in the first two forms is of the simplest character.

According to Brefeld's figures, one can see that the conidia vary considerably in shape and size, some being long and narrow, others oval or nearly spherical. Some are exceedingly small, others very large. Lack of uniformity in shape and size is as striking as in case of the conidia of Cladosporium, for example. The conidial stage of Pilacre is to be looked upon as a separate and distinct spore form in the life history of

the fungus. Since conidia develop abundantly on agar cultures and on pieces of sterilized beech branches in test tubes, it is to be expected that this stage will be found in nature if one looks for it at the right time.

TAXONOMIC AND NOMENCLATORIAL RÉSUMÉ

This fungus, like most others, has had several generic and specific names applied to it. So far as known at present it was first described by Fries (*13*) in 1818 as *Onygena faginea*. Fries placed the fungus among the Gasteromycetes. This, however, is of little significance when we consider his conception of that group, which is indicated by the fact that he included in it not only true puff balls but also many Myxomycetes, Hyphomycetes, Discomycetes and even Agarics, for example, Nyctalis. The chief character upon which he relied, apparently, in determining relationship in these cases was the production of a powdery mass of spores of some kind.

There seems to be no doubt in regard to the fungus described by Fries as *Onygena faginea*, as the writers are informed by Professor Juel of Upsala, Sweden, who has kindly searched Fries's herbarium for them that there are several specimens of this species to be found there. One was sent by Schweinitz (*34, p. 65*) under the name *Onygena decorticata* Pers. and is mentioned by Fries (*16, p. 209*) as *O. faginea* Fr. and not Persoon's species (*29, p. 72*). The writers have examined duplicates of this gathering from Schweinitz's collection and find them to be typical *Pilacre faginea* (Fr.) B. and Br. There is also a specimen from Ohio in Fries's herbarium sent by Berkeley under the same name, and a Swedish specimen, which Professor Juel says "probably ought to be considered as the type of the species *Pilacre faginea*. It is labeled in Fries's hand "*Ecchyna faginea* Fr. Nobilissima Femsjo." Professor Juel says (in litt.) of this specimen "as far as I can see it is a typical specimen of what has been called "*Pilacre petersii* or *P. faginea* which I suppose to be synonymous."

It requires very little study of this fungus to show that it does not belong in the genus Onygena of Persoon, the type of which is the common discomycete *O. equina*, found on decaying hoofs and horns of animals. The generic name Onygena is now generally accepted and applied to the discomycete and probably no one would now suggest applying it to *Pilacre faginea*.

Fries's first description of the genus Pilacre was written in 1825 (*15, p. 364*). This description is too general and indefinite to connect it with any specific fungus at present. At the end of the description *Stilbum incarnatum* Weinmann (in litt.) is cited. Just what plant Fries had from Weinmann under this name no one has yet determined. Professor Juel has kindly searched through Fries's herbarium for the specimen of this from Weinmann but has been unable to find it under Pilacre or any of the various generic names which have at one time or another been suggested in connection with it; for example, Ecchyna, Onygena, Stilbum, and Roesleria. Professor Juel thinks it probable that the plant referred to was a lichen and the description certainly suggests Coniocybe or Calicium. He finds under Calicium, specimens from Weinmann but nothing labeled *incarnatum* or *Weinmannii*. Fries, in 1829 (*16, p. 204*), renamed the fungus, dedicating it to the collector, Doctor Weinmann of Petrograd. Dr. de Jaczewski of Petrograd has kindly made a search for Weinmann's collections. In a letter just received he says: "About the collections of Weinmann, I am sorry to say there is nothing left, neither at the Academy nor anywhere else." This species is therefore indeterminable and must be abandoned.

Boudier (*4*) discussed Pilacre and asserted that it is synonymous with Roesleria of Von Thümen. He based his conclusion primarily on the supposition that *P. weinmannii* Fr., the original species of the genus, is identical with *Roesleria hypogea* Thüm. There is nothing in the original description of Fries to justify such a conclusion and he gives no evidence of having seen the type or authentic specimens. It seems far more probable that the original specimen of Weinmann was a lichen, as already mentioned and suggested by Professor Juel. The original name *incarnatum* of Weinmann alone would seem to preclude the possibility of the fungus being Roesleria, which has no suggestion of such color in any stages of development. If further evidence were desired, it would be found in the statement that *incarnatum* was found growing on bark while *Roesleria hypogea* always, so far as known, grows underground on roots.

Fries's last mention of the genus was in 1849 (*17, p. 361*) where he gives a generic description only and says "Cfr. Weinm, Ross," referring to Weinmann's paper (*37*). Since Fries's monotype of the genus can not be determined and since no other species were ever referred

to it by him, it would seem that the name should be abandoned or a type for it sought elsewhere.

Weinmann (*37*) referred two more species to the genus Pilacre, *P. subterranea* and *P. friesii*. These species are now both regarded by some authors as being synonomous with the Discomycete *Roeslerea hypogea* Thüm. et Pass (see Beckwith, *2*). In 1834 Weinmann (*38*) transferred his *Pilacre friesii* of 1832 to the genus Onygena and described another species, which he called *Pilacre friesii* and which, according to the original description, might be regarded as a synonym of *Pilacre faginea*. All these species of Weinmann are somewhat doubtful, as no type or authentic specimens have been seen or identified by recent authors.

It is very clear from the description of this genus and the species referred to, that the authors had very little definite knowledge of the structure and relationships of the specimens with which they were dealing. It will be noted from the account given above of Fries's treatment of his genus Pilacre that he never referred to it his *Onygena faginea*. This was first referred to the genus by Berkeley and Broome in 1850 (*3, p. 365*). It is clear from Fries's treatment of *O. faginea* that he was more or less doubtful from the beginning as to its relationship, and finally in 1857 (*19, p. 151*) decided to call it *Ecchyna faginea*.

The history of the genus Ecchyna also show how unsatisfactory his ideas of the application and limits of some of the names he proposed were, especially when they related to the smaller fungi, a real knowledge of which required a more or less careful microscopic study. Fries proposed this name Ecchyna in 1819 (*14, p. 80*), giving the name only and adding "l. c." What the "l. c." refers to the writers have been unable to determine. The first work cited above on the page contains no mention of this name so far as they can discover, nor have they been able to find mention of it in any of the earlier works referred to by him in the publication cited.

The next mention of this name by Fries, in 1825 (*15, p. 151*), is in an observation under the description of Onygena of Persoon, in which he says he finds a notable fungus with hornlike branches but without heads, which he has called Ecchyna, but it may be a monstrous form of *O. faginea*. His next mention of this generic name is in 1849 (*17, p. 446*) in a footnote in Latin under *O. faginea*, which we translate as follows: "There exists intermediate between this and *Onygena faginea* and the next [Lasioderma] a fungus provisionally named Ecchyna." A brief discussion follows in which he says "it is without asci."

His next reference to this genus is in 1851 (*18, p. 135*) in which he says in Latin:

> I have proposed Ecchyna for *Onygena faginea* only as a subgenus of Onygena. It is very different from Pilacre to which my friend Berkeley refers it. It might be justifiable to propose *Onygena faginea* as an entirely distinct genus, but however others may feel it is impossible for me, trusting to the harmony of nature to attribute great weight to microscopical characters which are easily interpreted by morphological and physiological laws.

This incidentally throws interesting light upon Fries's mode of reasoning and interpreting nature. Fries's next mention of this species is in 1857 (*19, p. 151*) where he simply gives the name in a list of the species of Gasteromycetes occurring in central Sweden. Whether he was still in doubt as to the full generic rank of the name is not indicated. It is, therefore, difficult to say when, if ever, the name was really proposed as a genus by Fries. Notwithstanding this, Patouillard, 1900 (*28, p. 37*), takes up Ecchyna as a valid generic name citing Fries's footnote of 1849 as the place of publication.

If the generic name of this fungus is to be selected on the basis of priority of publication, there are at least two much older names than Ecchyna, the application of which is undoubted. The first of these is Phleogena Link, 1833 (*24, p. 396*). Link described the genus and then the monotype, *Phleogena faginea* and cites *Onygena faginea* Fries, as a synonym. This name appears to have been entirely neglected and is the oldest valid name known at present according to the priority rule.

In 1854 (*9, p. 47*) Corda, recognizing that *Onygena faginea* Fries, was very different from the true Onygena of Persoon, gave the species another generic name, Botryochaete. Corda apparently overlooked or disregarded the fact that Link had already proposed a generic name for the same species. Neither of these names ever came into general use.

Berkeley and Broome in 1850 (*3, p. 365*) first referred *Onygena faginea* of Fries to the genus Pilacre, and this combination has been generally used ever since. The same fungus was described by Berkeley and Curtis in 1859(*3, v. 3, p. 362*) from American specimens collected by Peters in Alabama and named *Pilacre petersii*. Saccardo in his Sylloge (*32, p. 362*) has referred ten species to Pilacre. Of these *P. friesii* Weinmann and *P. petersii*, B. & C. are apparently both synonyms of *P. faginea*, the latter certainly so. The other species mentioned are unknown to the writers; they can not

therefore say whether these are congeneric or not. *P. pallida*, E. & E. (*12*, *p. 59*) is a very different fungus, as the writers have determined by an examination of some of the original material upon which the species was based, and is certainly not congeneric with *P. faginea*.

In view of the fact as already indicated, that the original type of the genus Pilacre of Fries is unknown and that the name has been generally applied for the last 74 years to the fungus originally named *Onygena faginea* by Fries the writers propose to adopt Pilacre on the basis of general usage and assign to it as its type the common and well known species *P. faginea*, with which it has been so long associated. This procedure, they believe, will best serve the primary purposes and aims of binomial nomenclature, by fixing once for all with certainty the application of this well-known and established name, thus avoiding the necessity for any change in the future. It may be of interest to note that Möller (*26*, *p. 54*) arrived at the same conclusion in regard to the application of this generic name.

The synonomy of this species so far as known at present is as follows:

Onygena faginea Fr. 1818 (*13*, *p. 25.*)
Onygena decorticata Schw. 1822 (*34*, *p. 65.*) (Not *O. decorticata* Pers. 1799. *29.*)
Phleogena faginea (Fr.) Link 1833 (*24*, *p. 396.*)
Pilacre friesii Weinm. in Linnaea 1834 (*38*, *p. 413–414.*) (Not *P. friesii* Weinm., in Flora, 1832. *37*, *p. 458.*)
Pilacre faginea (Fr.) Berk. & Br. 1850 (*3*, *p. 365.*)
Botryochaete faginea (Fr.) Corda 1854. (*9*, *p. 46.*)
Ecchyna faginea Fr. 1857 (*18*, *p. 151.*)
Pilacre petersii Berk. & Curt. 1859 (*3*, *p. 382.*)

Stilbum pilacreforme Rich. 1881 (*30*) and 1882 (*31*, *p. 241*), cited as a synonym by Beckwith (*2*), does not appear, according to Richon's description, to be Pilacre. However, the writers have not seen Richon's specimens and so can not speak positively.

MORPHOLOGY AND RELATIONSHIP

As already stated, Fries (*13*) referred this fungus to the Gasteromycetes. This, however, has little significance on account of his lack of knowledge of the morphology and development of the fungus and also as it was the result of the most superficial observation. Neither Link nor Corda, both of whom renamed the fungus, added any thing of value to our knowledge of it. Corda (*9*, *p. 46*), however, did consider it a basidiomycete and placed it next to the hypogaeous Gasteromycete, Melanogaster. He says that the family to which it belongs is doubtful and the genus is of uncertain affinity, but emphasizes the fact that it has basidia although he stated that they are 1- to 4-spored.

The next writer to consider the relationship of this fungus was Tulasne (*35*, *p. 294–295*). He first regarded Pilacre as related to Ptychogaster, the conidial or chlamydospore condition of a Polyporus. Later (*36*, *p. 228*) he investigated the spore formation of Pilacre and made another interpretation comparing it to *Hypochnus purpureus* and regarding it as related to the Auriculariae. De Bary (*1*, *p. 335*) does not seem to have made any particular study of this fungus and gives Tulasne's earlier interpretation of it. The next contribution to our knowledge of this plant was made by Schroeter in 1877 (*33*). In connection with a discussion of the systematic position of Tulostoma, he mentions Tulasne's studies of Pilacre and states that according to his illustrations of the spore formation, these two fungi are very similar, and he says that instead of being closely related to Hypochnus, he regards it as much more closely related to Tulostoma, and concludes with the statement: "I regard it as apparent that this fungus, Pilacre, which has heretofore been placed in different families, is the type as already noted of a second genus of the family Tulostomaceae."

It remained for Brefeld to give us the first clear and accurate account of the life history of this fungus. He grew it in culture and obtained a conidial condition which is a Hyphomycete similar in general structure and appearance to Rhinotrichum or Haplaria. He also gave detailed illustrations of its perfect condition with the typical transversely divided four-celled basidia and basidiospores. He regarded the fungus as closely related to the Auriculariales at first, but in his later summary or review of his work he mentions the similarity between the basidia of Pilacre and Tulostoma (*7*, *p. 197*) and also mentions the general resemblance of the fruiting bodies, both being angiocarpous; in his final arrangement of the fungi he places Pilacre intermediate between the Auriculariaceae and Lycoperdaceae. His idea in regard to the relation between the conidial form and the perfect stage and its significance as to the development of the Basidiomycetes from the Hyphomycetes is, of course, now generally regarded as untenable.

Möller (*26*, *p. 48–64*), one of Brefeld's students, was the next to treat of this fungus and some of its relatives. He found in Brazil a fungus which he re-

garded as identical with *Pilacre petersii* of Europe. He was, however, unable to germinate the spores of the fungus and so did not obtain pure cultures. He found, however, other fungi which he regarded as belonging to the Protobasidiomycetes and related to Pilacre. One especially interesting belongs to the genus Pilacrella, differing from Pilacre in having a much looser head without a distinctive peridium, but producing prolonged branching hyphae all about the head. This fungus also produces a conidial form of hyphomycetous character entirely distinct from the basidiomycetous fructification. The conidia are sometimes borne laterally on the four terminal cells of the hyphae, suggesting somewhat in appearance the basidia. This led Möller to consider this fungus as showing a perfect transition between the Hyphomycetes and the Basidiomycetes. Möller regarded Pilacre as being most closely related to Stypinella, a genus of Auriculariaceae. No recent additions have been made to our knowledge of Pilacre.

Lindau (*23, p. 86*) places the genus Pilacre in the Auriculariaceae. Saccardo (*32, p. 579*) places it in the Stilbaceae of the Hypomycetes, but speaks of its possible relation to Roesleria and its lack of a typical hyphomycetous character. He includes 10 species, most of which have been described by Berkeley and Broome and Berkeley and Curtis. These are all from the Tropics and very little is known in regard to their structure or relationship.

Bayliss-Elliott and Grove (*11*) also discussed the fungus and regarded it as probably the conidial condition of Roesleria. Overholts, 1922 (*27, p. 165*), says he was also able to find the cross walls in the basidia, which are characteristic of the Auriculariales; but says that the fungus departs widely in consistency and habit from other members of the order. Lloyd (*25*) in a recent reference to the fungus considers it a hyphomycete.

Beckwith (*2*) has pointed out that many authors have confused Pilacre with Roesleria and Coniocybe, some claiming that *Pilacre petersii* is merely a conidial stage of Roesleria. She has shown by pure cultures that Roesleria develops only ascocarps in culture.

As a result of the writers' studies of this fungus and those cited bearing on this and other fungi which are more or less related to it, they are of the opinion that it represents what might be appropriately called a protogasteromycete. Its nearest known relative at present among the Gasteromycetes is apparently Tulostoma, as suggested by Schroeter (*33*). It is not unlikely that when the fungi of the world are more fully known other genera will be found partially bridging the gap between genera like Pilacre and Tulostoma.

As already indicated, Brefeld and Möller showed that *Pilacre faginea* possesses true basidia, and on account of these being transversely septate and bearing lateral spores regarded it as more closely related to the Auriculariales than to the Gasteromycetes. Brefeld (*5, p. 194*) at first, basing his opinion on Tulasne's account of Pilacre, stated that the Gasteromycetes had most probably arisen from a similar form. Schroeter pointed out its angiocarpous character and the resemblance of the basidia and spore formation to that of Tulostoma.

Eidam (*10*) has shown the presence of clamp connections in Cyathus and Crucibulum, while so far as the writers know at present, these have not been found in the Auriculariales. This would also appear to indicate the closer relationship of Pilacre to the Gasteromycetes than to the Auriculariales.

SUMMARY

The life history of *Pilacre faginea* as determined by Brefeld has been verified by pure cultures from basidiospores and also from conidia, the perfect or basidiomycetous stage being produced in both cases. It is shown that this fungus has true basidia, each arising from a clamp connection, which is a two-nucleate structure. These nuclei fuse and undergo two divisions so that the mature basidium consists of four uninucleate cells.

The fungus is shown to possess two distinct stages of development, the gametophytic and sporophytic, differing in the color and character of the mycelium and in the character and method of production of the spores. The conidial stage (gametophytic) has the general appearance of a hyphomycete similar to Rhinotrichum or Haplaria. The basidial stage (sporophytic) has the general characteristics of a Basidiomycete, having clamp connections and uniformly four-spored basidia. The sporophytic stage produces a distinct more or less stipitate sporocarp having a gleba and peridium suggestive of a Gasteromycete. Brefeld and Möller, who made careful studies of the fungus, regarded it as intermediate between the Auriculariales and Gasteromycetes. Schroeter regarded it as having closer affinities with the Gasteromycetes.

The evidence accumulated by the writers leads them to regard this fungus

as a Protogasteromycete, whose nearest known relative among the puff balls appears to be Tulostoma.

The history of the nomenclature of this fungus shows a difference of opinion as to the application of the generic name Pilacre. There seems to be no good reason for regarding it as a synonym of Roesleria, as the original type of the genus is unknown and undetermined.

The writers adopt and recommend the application of the generic name Pilacre to *P. faginea* as the nomenclatorial type of the genus because this name has been established and in general use for this fungus for the past 74 years.

It is shown that this fungus has no relation to Roesleria, the Discomycete which some authors have considered as a perfect stage of *Pilacre faginea*.

LITERATURE CITED

(1) BARY, A. DE
1887. COMPARATIVE MORPHOLOGY AND BIOLOGY OF THE FUNGI, MYCETOZOA AND BACTERIA. 525 p., illus. Oxford.

(2) BECKWITH, A. M.
1924. THE LIFE HISTORY OF THE GRAPE ROOT ROT FUNGUS, ROESLERIA HYPOGAEA THÜM. ET PASS. Jour. Agr. Research 27: 609-617, illus.

(3) BERKELEY, M. J., and BROOME, C. E.
1850-59. NOTICES OF BRITISH FUNGI. Ann. and Mag. Nat. Hist. (II) 5: 365-380; (III) 3: 356-377, illus.

(4) BOUDIER, E.
1888. NOTE SUR LE VRAI GENRE PILACRE ET LA PLACE QU'IL DOIT OCCUPER DANS LES CLASSIFICATIONS. Jour. Bot. [Paris] 2: 261-264.

(5) BREFELD, O.
1877. BASIDIOMYCETEN I. 226 p., illus. Leipzig. (*His* Botanische Untersuchungen über Schimmelpilze, Heft 3.)

(6) ———
1888. BASIDIOMYCETEN II. 178 p., illus. Leipzig. (*His* Untersuchungen aus dem Gesammtgebiete der Mykologie, Heft 7.)

(7) ———
1889. BASIDIOMYCETEN III. 305 p., illus. Leipzig. (*His* Untersuchungen aus dem Gesammtgebiete der Mykologie, Heft 8.)

(8) ———
1908. DIE KULTUR DER PILZE. 256 p. Münster. (*His* Untersuchungen aus dem Gesammtgebiete der Mykologie, Bd. 14.)

(9) CORDA, A. C. J.
1854. ICONES FUNGORUM. t. 6, illus. Pragae.

(10) EIDAM, E.
1876. DIE KEIMUNG DER SPOREN UND DIE ENTSTEHUNG DER FRUCHT-KÖRPER BEI DEN NIDULARIEEN. Cohn, F., Beiträge zur Biologie der Pflanzen 2: 221-248, illus.

(11) ELLIOTT, J. S. BAYLISS, and GROVE, W. B.
1916. ROESLERIA FALLIDA, SACC. Ann. Bot. 30: 407-414, illus.

(12) ELLIS, J. B., and EVERHART, B. M.
1900. NEW SPECIES OF FUNGI FROM VARIOUS LOCALITIES WITH NOTES ON SOME PUBLISHED SPECIES. Bul. Torrey Bot. Club 27: 49-64.

(13) FRIES, E. M.
1818. SYMBOLAE GASTEROMYCETUM AD ILLUSTRANDUM FLORAM SUECICAM. 3: 17-25. [Diss. acad.]. Lundae. [Not seen. Reference from Lindau, G., and Sydow, P., Thesaurus Litt. Mycol. 1: 494. 1908.]

(14) ———
1819. NOVITIAE FLORAE SVECICAE. 80 p. Lundae.

(15) ———
1825. SYSTEMA ORBIS VEGETABILIS. 374 p. Lundae.

(16) FRIES, E. M.
1829. SYSTEMA MYCOLOGICUM. v. 3. Gryphiswaldiae.

(17) ———
1846. SUMMA VEGETABILIUM SCANDINAVIAE. 572 p. Holmiae & Lipsiae.

(18) ———
[1851?] NOVAE SYMBOLAE MYCOLOGICAE. Nova Acta Reg. Soc. Sci. Upsal. (III) 1: 17-136. 1855.

(19) ———
1857. SVAMPARNES CALENDARIUM UNDER MEDLERSTA SVERIGES HORISONT. Öfvers. K. Vetensk. Akad. Förhandl. 14: 137-155.

(20) GILBERT, E. M.
1921. CYTOLOGICAL STUDIES OF THE LOWER BASIDIOMYCETES. Trans. Wis. Acad. Sci., Arts and Letters 20: 387-397, illus.

(21) JUEL, H. O.
1898. STILBUM VULGARE TODE. EIN BISHER VERKANNTER BASIDIOMYCET. K. Svenska Vetensk. Akad. Handl., Bihang. Bd. 24, Afd. 3, no. 9, 15 p., illus.

(22) KNIEP, H.
1917. BEITRÄGE ZUR KENNTNIS DER HYMENOMYCETEN. Ztschr. Bot. 9: 81-118, illus.

(23) LINDAU, G.
1900. AURICULARIALES. Engler, A., and Prantl, K., Die natürlichen Pflanzenfamilien. 1 (1**): 82-88, illus.

(24) LINK, H. F.
1833. HANDBUCH ZUR ERKENNUNG DER NUTZBARSTEN UND AM HÄUFIGSTEN VORKOMMENDEN GEWÄCHSE. Theil 3. Berlin.

(25) LLOYD, C. G.
1923. NOTE 1145.—PILACRE FAGINEA FROM D. S. WELCH, NEW YORK. Mycolog. Notes 7: 1207.

(26) MÖLLER, A.
1895. PROTOBASIDIOMYCETEN. 179 p., illus. Jena. (Schimper, A. F. W., Botanische Mittheilungen aus den Tropen, Heft 8.)

(27) OVERHOLTS, L. O.
1922. MYCOLOGICAL NOTES FOR 1920. Bul. Torrey Bot. Club 49: 165-173, illus.

(28) PATOUILLARD, N.
1900. ESSAI TAXONOMIQUE SUR LES FAMILLES ET LES GENRES DES HYMÉNOMYCÈTES. 184 p., illus. Lons-le-Saunier.

(29) PERSOON, C. H.
1799. OBSERVATIONES MYCOLOGICAE. v. 2., illus. Lipsiae et Lucernae.

(30) RICHON, C.
1881. ÉTUDE SUR LE VIBRISSEA HYPOGAEA ET SON ÉTAT CONIDIAL. (Compt. Rend. Comité de vigilance contre le phylloxera pour le départm. de la Marne, 18 août et décemb. 1881) tab. [Not seen. Reference from Lindau, G., and Sydow, P., Thesaurus Litt. Mycol. 2: 367. 1909.]

(31) ———
1882. SUR LE VIBRISSEA HYPOGAEA ET LA GODONRIA MÜHLENBECKII. Bul. Soc. Bot. France 29: 240-243, illus.

(32) SACCARDO, P. A.
1886. SYLLOGE FUNGORUM. v. 4. Patavii.

(33) SCHROETER, J.
1877. NUEBER DIE ENTWICKELUNG UND DIE SYSTEMATISCHE STELLUNG VON TULOSTOMA PERS. Cohn, F., Beiträge zur Biologie der Pflanzen 2: 65-72.

(34) SCHWEINITZ, L. D. VON
[1822]. SYNOPSIS FUNGORUM CAROLINAE SUPERIORIS, SECUNDUM OBSERVATIONES. 105 p., illus. [n. p.]

(35) TULNASNE, L. R.
1865. NOTE SUR LE PTYCHOGASTER ALBUS, COES. Ann. Sci. Nat. Bot. (V) 4: 290-296.

(36) ———
1872. NOUVELLES NOTES SUR LES FUNGI TREMELLINI ET LEURS ALLIÉS. Ann. Sci. Nat. Bot. (V) 15: 215-235, illus.

(37) WEINMANN, J. A.
1832. OBSERVATIONES QUAEDAM MYCOLOGICAE AD FLORAM PETROPOLITANAM SPECTANTES. Flora 15: 449-460.

(38) ———
1834. ENUMERATIO GASTEROMYCETUM GENUINORUM HUC USQUE IN IMPERIO RUTHENICO OBSERVATORUM. Linnaea 9: 403-416.

TOXICITY STUDIES WITH DICYANODIAMIDE ON PLANTS[1]

By F. E. Allison, *Biochemist, Fixed Nitrogen Research Laboratory*, J. J. Skinner, *Biochemist*, and F. R. Reid, *Assistant Biochemist, Soil-Fertility Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The conclusions reached from the experimental work dealing with the effect of dicyanodiamide on plant growth which have been reported by various investigators vary widely. In some instances the compound has been reported as very toxic to plants, in others as merely unavailable as a nitrogen source for plants, and practically inert. At the Fixed Nitrogen Research Laboratory of the United States Department of Agriculture as well as elsewhere, experiments have shown that small quantities of dicyanodiamide prevent nitrification in the soil. It seemed, therefore, that possibly the bad effects of the compound might be due to the fact that in its presence the soil nitrogen is not converted into nitrates or at least is converted so slowly that plants undergo nitrate starvation. Thus dicyanodiamide would act as an indirect rather than direct poison to plants. The experiments reported here were planned to determine this point by studying the growth of plants in soils containing dicyanodiamide and varying quantities of sodium nitrate.

SOILS AND PLANTS USED IN THE EXPERIMENTS

The soils used were Norfolk sandy loam, a Coastal Plain soil obtained from near Norfolk, Va., and Chester loam, a Piedmont Plateau soil secured from near McLean, Va. The plants used were wheat and cowpeas.

SOIL PREPARATION AND FERTILIZATION

The materials to be tested were thoroughly mixed with each 10-pound portion of sieved soil and put into 1-gallon glazed pots and planted, each treatment being in duplicate. After germination was complete the plants were thinned to 10 and 5, respectively, for wheat and cowpeas.

All pots except those designated as "no fertilizer" received 80 pounds of P_2O_5 and 40 pounds of K_2O per acre in the form of monocalcium phosphate and potassium sulphate, respectively. The calculations were based on 2,000,000 pounds of soil per acre to a depth of 6 inches. The sodium nitrate and dicyanodiamide were applied at various rates and in varying proportions. Check pots were included in which the normal applications of phosphate and potash were made, but no nitrogen. In the tables which follow, the term fertilizer ratio refers to the ratio of NH_3, P_2O_5, and K_2O in the order named.

DURATION OF EXPERIMENTS

The wheat was planted on March 18, 1922, and cut on May 1, 1922. During this period the pots were weighed at frequent intervals and the water lost by evaporation restored. The wheat was harvested sooner than desired because of an unusually severe attack of mildew.

The cowpeas were planted on May 16, 1922, and cut on June 21, 1922. The excessive heat in the greenhouse was causing many of the lower leaves to drop, making necessary the early termination of the experiment.

EXPERIMENTAL RESULTS

First Experiment

RESULTS WITH WHEAT

A. Sodium Nitrate Constant, Dicyanodiamide Variable

The results from pots of wheat treated with varying quantities of dicyanodiamide, together with a constant and adequate nitrate supply, are given in Table I and Figure 1. Tests were also included using sodium nitrate and dicyanodiamide singly for comparison.

[1] Received for publication June 5, 1924; issued May, 1925. The experiments herein reported were conducted at Arlington, Va., the Soil Fertility greenhouses being used.

These figures show that dicyanodiamide at the high rate of 40 pounds of ammonia per acre is only slightly toxic to wheat under the conditions used. On the sandy Norfolk soil the decrease in weight of the plants amounted to approximately 17 per cent and on the Chester loam to 13 per cent, as compared with the control. Where sodium nitrate was used together with different quantities of dicyanodiamide, the green weights were practically as large with the dicyanodiamide as without in the case of the lighter soil. The dicyanodiamide did cause a slight drop at the highest rate, but this was almost within experimental error. On the Chester loam the results were similar, except that both 20 and 40 pounds of ammonia as dicyanodiamide used with sodium nitrate caused slight decreases as compared with nitrate alone. These results seem to show that dicyanodiamide is toxic to wheat to a slight extent at the high rate used but can not be considered as a marked direct poison. It did not greatly lessen the fertilizing value of sodium nitrate or greatly affect the growth of the plants.

The observations made during growth of the wheat corresponded rather closely to the final green weights. Dicyanodiamide caused a slight tip-burning at all rates of application on the Norfolk sandy loam, the burning being greatest when the plants were about 2 weeks old. There was a partial recovery later. At the rates of 20 and 40 pounds of ammonia, many of the leaf tips were burned back 2 to 3 inches. The rate of growth did not appear to be greatly affected, however. The burning effect was not lessened by application of sodium nitrate. On the Chester loam there was practically no burning at any time.

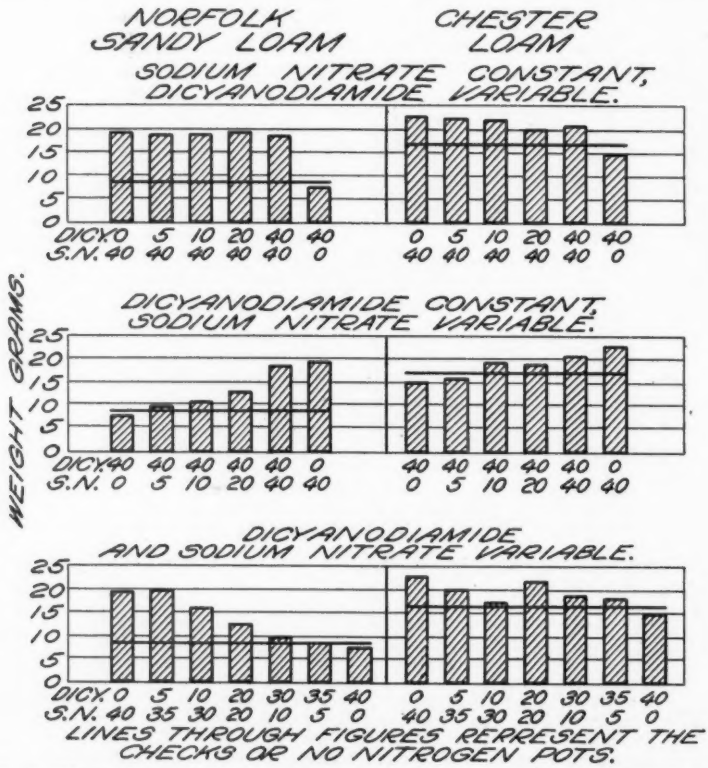

NORFOLK SANDY LOAM
CHESTER LOAM
SODIUM NITRATE CONSTANT, DICYANODIAMIDE VARIABLE.
DICY. 0 5 10 20 40 40
S.N. 40 40 40 40 40 0
0 5 10 20 40 40
40 40 40 40 40 0
DICYANODIAMIDE CONSTANT, SODIUM NITRATE VARIABLE.
DICY. 40 40 40 40 40 0
S.N. 0 5 10 20 40 40
40 40 40 40 40 0
0 5 10 20 40 40
DICYANODIAMIDE AND SODIUM NITRATE VARIABLE.
DICY. 0 5 10 20 30 35 40
S.N. 40 35 30 20 10 5 0
0 5 10 20 30 35 40
40 35 30 20 10 5 0
WEIGHT GRAMS.
0 5 10 15 20 25
LINES THROUGH FIGURES REPRESENT THE CHECKS OR NO NITROGEN POTS.

FIG. 1.—Diagram showing the yields of wheat from pots receiving various amounts of dicyanodiamide and sodium nitrate

TABLE I.—*Green weights of wheat from pots receiving various quantities of dicyanodiamide in the presence of a constant nitrate supply*

Treatment *	Fertilizer ratio NH_3-P_2O_5-K_2O	Norfolk sandy loam			Chester loam		
		Green weight	Average	Increase or decrease over check	Green weight	Average	Increase or decrease over check
		Grams			*Grams*		
No fertilizer	0-0-0	8.4 8.0	8.2		12.7 12.6	12.7	
No nitrogen	0-8-4	9.2 9.0 9.3 8.0 9.2 8.5	8.9		16.3 15.8 14.4 17.9 22.1 13.9	16.7	
Sodium nitrate 40	4-8-4	18.2 17.8 18.9 21.7	19.2	10.3	22.4 21.4 23.0 24.2	22.8	6.1
Dicyanodiamide 40	4-8-4	6.8 7.9	7.4	−1.5	14.9 14.3	14.6	−2.1
Sodium nitrate 40, dicyanodiamide 5	4.5-8-4	18.8 18.9	18.9	10.0	22.8 21.5	22.2	5.5
Sodium nitrate 40, dicyanodiamide 10	5-8-4	18.4 19.4	18.9	10.0	21.2 22.8	22.0	5.3
Sodium nitrate 40, dicyanodiamide 20	6-8-4	20.4 18.0	19.2	10.3	21.5 18.4	20.0	3.3
Sodium nitrate 40, dicyanodiamide 40	8-8-4	17.6 19.0	18.3	9.4	20.6 20.4	20.5	3.8

* In this table and those which follow the figures refer to pounds of nitrogen per acre expressed as NH_3 (2,000,000 pounds of soil).

TABLE II.—*Green weights of wheat from pots receiving various quantities of sodium nitrate in the presence of dicyanodiamide*

Treatment	Fertilizer ratio NH_3-P_2O_5-K_2O	Norfolk sandy loam			Chester loam		
		Green weight	Average	Increase or decrease over check	Green weight	Average	Increase or decrease over check
		Grams			*Grams*		
No fertilizer	0-0-0		8.2			12.7	
No nitrogen	0-8-4		8.9			16.7	
Sodium nitrate 40	4-8-4		19.2	10.3		22.8	6.1
Dicyanodiamide 40	4-8-4	6.8 7.9	7.4	−1.5	14.9 14.3	14.6	−2.1
Dicyanodiamide 40, sodium nitrate 5	4.5-8-4	10.2 8.9	9.6	.7	16.0 15.7	15.9	−.8
Dicyanodiamide 40, sodium nitrate 10	5-8-4	9.5 10.9	10.2	1.3	18.9 19.1	19.0	2.3
Dicyanodiamide 40, sodium nitrate 20	6-8-4	11.6 13.6	12.6	3.7	18.7 18.7	18.7	2.0
Dicyanodiamide 40, sodium nitrate 40	8-8-4	17.6 19.0	18.3	9.4	20.6 20.4	20.5	3.8

B. Dicyanodiamide Constant; Sodium Nitrate Variable

The green weights of wheat from pots receiving dicyanodiamide at a rate equivalent to 40 pounds of ammonia per acre with 0 to 40 pounds of sodium nitrate are given in Table II and in Figure 1. The data show that in the presence of sodium nitrate dicyanodiamide continues to exert an injurious, although not very noticeable, effect on the plants. Considering the pots receiving dicyanodiamide alone

as the check, it will be observed that sodium nitrate produced an increase of growth in about the same proportion as would be expected where the only source of nitrogen was nitrate. On the sandy loam nitrate equivalent to 5 pounds of ammonia per acre was more than sufficient to counteract the 40 pounds of ammonia as dicyanodiamide. On the Chester loam this quantity was not quite sufficient, but 10 pounds was more than adequate.

C. Dicyanodiamide and Sodium Nitrate in Varying Proportions; Nitrogen Constant

This experiment was planned so as to keep the nitrogen content constant throughout while supplying the nitrogen as dicyanodiamide and sodium nitrate in different proportions. Such mixtures would approximate ordinary fertilizer practice on the basis of nitrogen content, but would contain a portion of the nitrogen as unavailable dicyanodiamide. The data given in Table III and shown in Figure 1 confirm the results reported in previous tables, again demonstrating that dicyanodiamide is not markedly toxic and that the reduction in green weight is overcome by the application of very small quantities of sodium nitrate. The yields obtained with mixtures of the two materials depended almost wholly on the nitrate content, particularly on the sandy soil. The yields on the Chester loam were somewhat irregular and not always in agreement with those to be expected from the fertilizer treatments.

RESULTS WITH COWPEAS

In order to determine the residual effects of dicyanodiamide applications, a few of the pots which had grown a crop of wheat were planted to cowpeas without additional treatment. The green weights of the plants are given in Table IV.

The results obtained on the Norfolk sandy loam show a marked injury by dicyanodiamide even to the second crop, in fact greater than to the first crop. However, there seems to be no doubt that the cowpea is much less tolerant of dicyanodiamide than is wheat. This point is well illustrated in subsequent tables. During growth the cowpeas showed the same leaf yellowing and mosaic appearance as where fresh applications of dicyanodiamide had been used, but possibly to a slighter degree. This is in agreement with the results reported by the majority of investigators which show that dicyanodiamide is not readily decomposed in the soil. Under greenhouse conditions, where there was no leaching, the dicyanodiamide would necessarily have to be broken up chemically in order for its injurious effects to be eliminated, unless perhaps a small amount might be removed from the soil solution by absorption.

On the Chester loam the results obtained with cowpeas were somewhat indefinite, the mixture of dicyanodiamide and sodium nitrate giving slightly smaller green weights than the check, whereas both dicyanodiamide alone and sodium nitrate alone gave increases over the no-nitrogen treatment. The differences are not very significant.

TABLE III.—*Green weights of wheat from pots receiving varying quantities of both dicyanodiamide and sodium nitrate*

Treatment	Fertilizer ratio NH_3-P_2O_5-K_2O	Green weight	Average	Increase or decrease over check	Green weight	Average	Increase or decrease over check
		Grams			*Grams*		
No fertilizer	0-0-0		8.2			12.7	
No nitrogen	0-8-4		8.9			16.7	
Sodium nitrate 40	4-8-4		19.2	10.3		22.8	6.1
Sodium nitrate 35, dicyanodiamide 5	4-8-4	20.3 19.3	19.8	10.9	21.4 18.6	20.0	3.3
Sodium nitrate 30, dicyanodiamide 10	4-8-4	16.4 15.5	16.0	7.1	18.0 16.0	17.0	.3
Sodium nitrate 20, dicyanodiamide 20	4-8-4	12.2 12.3	12.3	3.4	21.5 22.1	21.8	5.1
Sodium nitrate 10, dicyanodiamide 30	4-8-4	8.3 10.8	9.6	.7	17.6 20.2	18.9	2.2
Sodium nitrate 5, dicyanodiamide 35	4-8-4	7.8 9.2	8.5	−.4	19.1 17.1	18.1	1.4
Dicyanodiamide 40	4-8-4	6.8 7.9	7.4	−1.5	14.9 14.3	14.6	−2.1

TABLE IV.—*Green weight of cowpeas grown after wheat without additional fertilization*

NORFOLK SANDY LOAM

Source of nitrogen •	Fertilizer ratio	Green weight	Average	Increase or decrease over check	Nodule formation
		Grams			
No nitrogen	0-8-4	26.0	25.2		Abundant
		24.3			Do.
Sodium nitrate 40	4-8-4	25.4	25.4	0.2	Do.
		25.4			Do.
Dicyanodiamide 40	4-8-4	20.6	18.0	−7.2	Many.
		15.3			Do.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	18.3	17.5	−7.7	Do.
		16.6			Abundant.

CHESTER LOAM

No nitrogen	0-8-4	35.6	35.4		Few.
		35.1			Do.
Sodium nitrate 40	4-8-4	40.3	40.1	4.7	Many.
		39.9			Few.
Dicyanodiamide 40	4-8-4	38.7	37.4	2.0	Do.
		36.0			None.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	31.2	32.7	−2.7	Few.
		34.1			Do.

• The figures in this column refer to the quantities of nitrogen applied to the crop of wheat, previously grown in this soil.

In Table IV, as well as in those which follow, a column is included showing the relative abundance of nodules. No doubt the abundance of nodules on the plants in some pots, especially in those containing the Norfolk soil, was quite a factor in determining growth.

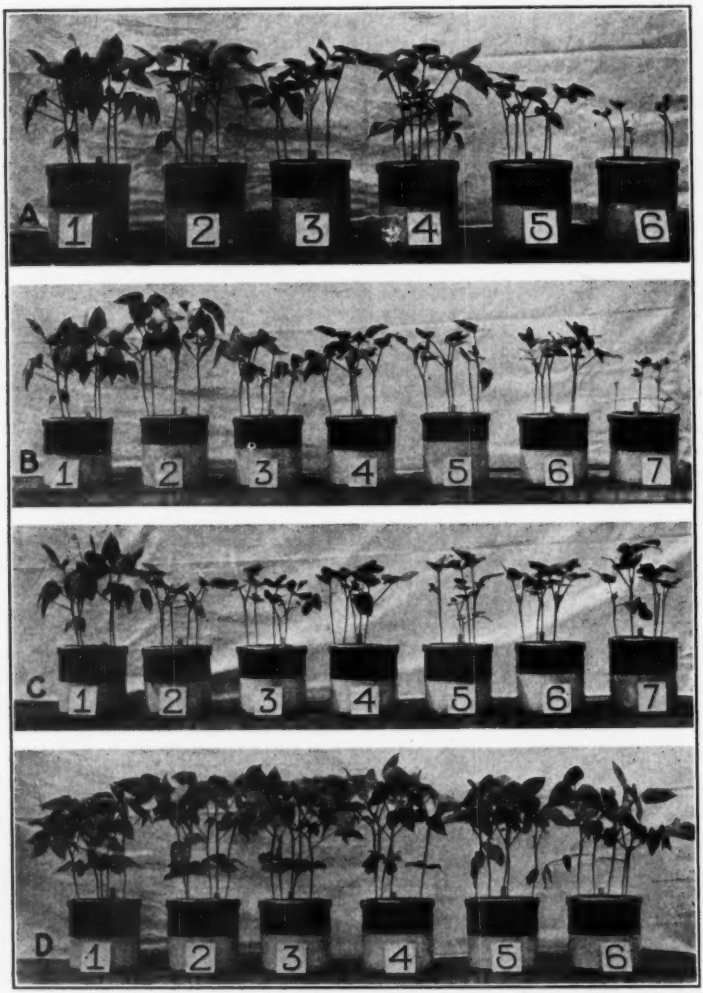
SECOND EXPERIMENT

EFFECT OF VARIOUS AMOUNTS OF DICYANODIAMIDE WITH AND WITHOUT SODIUM NITRATE ON COWPEAS

At the same time that the experiments with cowpeas reported on above were begun, another series was also included to determine the toxicity of dicyanodiamide to cowpeas and the relation of the nitrate supply to the injury. The results are given in Tables V and VI and illustrated in Figures 2 and 3. Plates 1 and 2 show the plants just prior to harvesting.

The results reported in Table V with the Norfolk sandy loam were somewhat unexpected. The soil which grew such a poor crop of wheat in the absence of a nitrogenous fertilizer showed negligible responses to sodium nitrate with cowpeas. Furthermore, small applications of dicyanodiamide depressed the yield quite markedly, while the larger quantities injured germination and practically killed the plants which did appear above the surface. In these extreme cases, the roots became brown and rotted. The use of sodium nitrate with the dicyanodiamide increased the injury up to 20 pounds of ammonia per acre and decreased it slightly thereafter. These results are decidedly different from those obtained with wheat.

The data for the Chester loam show a fair increase in green weight of the plants resulting from the use of sodium nitrate alone. Dicyanodiamide depressed the yields at 20 pounds of ammonia per acre and above. Where sodium nitrate was used with the dicyanodiamide the injury by the latter was partially overcome. The results obtained with cowpeas on this soil agreed more nearly with those obtained with wheat than was true with the lighter soil experiments. Nevertheless, the dicyanodiamide injury was greater for cowpeas than for wheat, as shown both by green weights and the yellowing and mosaic effects on the leaves.

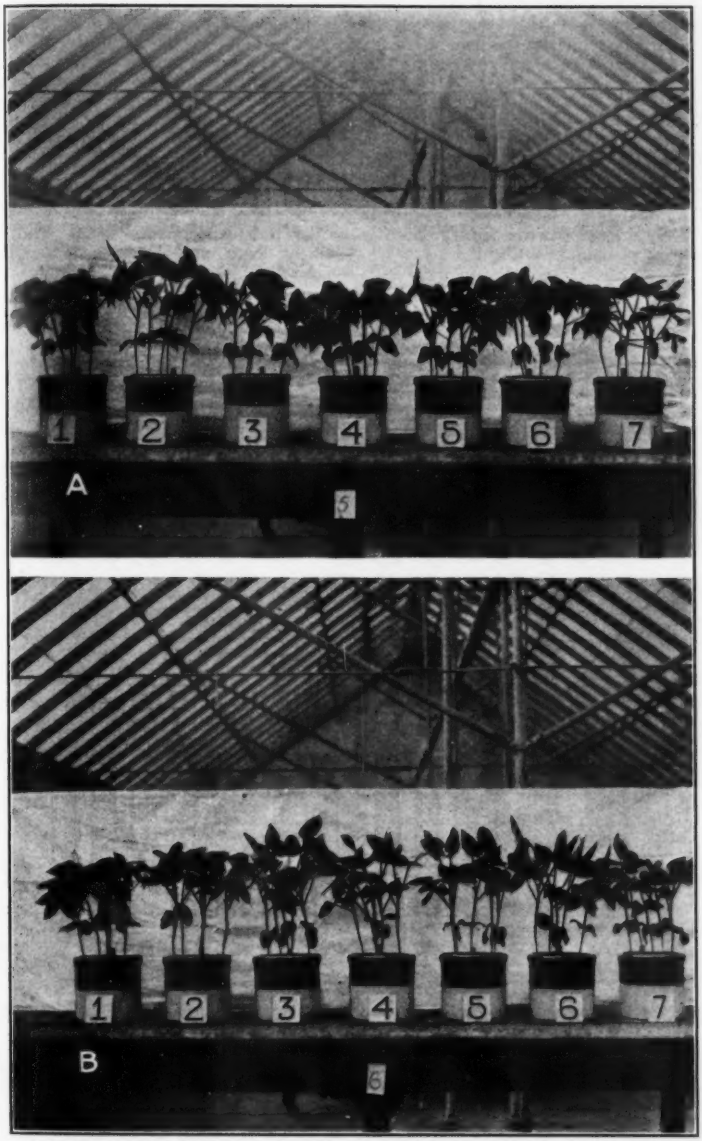


A.—Effect of dicyanodiamide on cowpeas grown on Norfolk sandy loam. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide, 5; 3, dicyanodiamide 10; 4, dicyanodiamide 20; 5, dicyanodiamide 40; 6, dicyanodiamide, 80.

B.—Growth of cowpeas on Norfolk sandy loam, receiving a constant quantity of sodium nitrate and varying quantities of dicyanodiamide. The applications are expressed in pounds NH_3 per acre. 1, no nitrogen; 2, sodium nitrate 40; 3, sodium nitrate 40, dicyanodiamide 5; 4, sodium nitrate 40, dicyanodiamide 10; 5, sodium nitrate 40, dicyanodiamide 20; 6 sodium nitrate 40, dicyanodiamide 40; 7, sodium nitrate 40, dicyanodiamide 80.

C.—Growth of cowpeas on Norfolk sandy loam receiving a constant quantity of dicyanodiamide and varying quantities of sodium nitrate. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide 40; 3, dicyanodiamide 40, sodium nitrate 5; 4, dicyanodiamide 40, sodium nitrate 10; 5, dicyanodiamide 40, sodium nitrate 20; 6, dicyanodiamide 40, sodium nitrate 40; 7, dicyanodiamide 40, sodium nitrate 80.

D.—Effect of dicyanodiamide on cowpeas grown on Chester loam. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide 5; 3, dicyanodiamide 10; 4, dicyanodiamide 20; 5, dicyanodiamide 40; 6, dicyanodiamide 80.



A.—Growth of cowpeas on Chester loam receiving a constant quantity of sodium nitrate and varying quantities of dicyanodiamide. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, sodium nitrate 40; 3, sodium nitrate 40, dicyanodiamide 5; 4, sodium nitrate 40, dicyanodiamide 10; 5, sodium nitrate 40, dicyanodiamide 20; 6, sodium nitrate 40, dicyanodiamide 40; 7, sodium nitrate 40, dicyanodiamide 80

B.—Growth of cowpeas on Chester loam receiving a constant quantity of dicyanodiamide and varying quantities of sodium nitrate. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide 40; 3, dicyanodiamide 40, sodium nitrate 5; 4, dicyanodiamide 40, sodium nitrate 10; 5, dicyanodiamide 40, sodium nitrate 20; 6, dicyanodiamide 40, sodium nitrate 40; 7, dicyanodiamide 40, sodium nitrate 80

19974—25†——3

EFFECT OF VARIOUS QUANTITIES OF SODIUM NITRATE WITH AND WITHOUT DICYANODIAMIDE ON COWPEAS

The data given in Table VI for the Norfolk sandy loam further emphasize the results reported in Table V. Applications of sodium nitrate had little effect on the growth of cowpeas either when used alone or with dicyanodiamide. In the latter case the nitrate usually increased the injury slightly instead of counteracting it.

The relative abundance of nodules on the roots of the cowpeas was not appreciably affected by the treatments, except that quantities of dicyanodiamide large enough to injure plant growth usually inhibited nodule formation. The untreated pots were usually the best inoculated.

SUMMARY

In pot experiments using Norfolk sandy loam and Chester loam, the

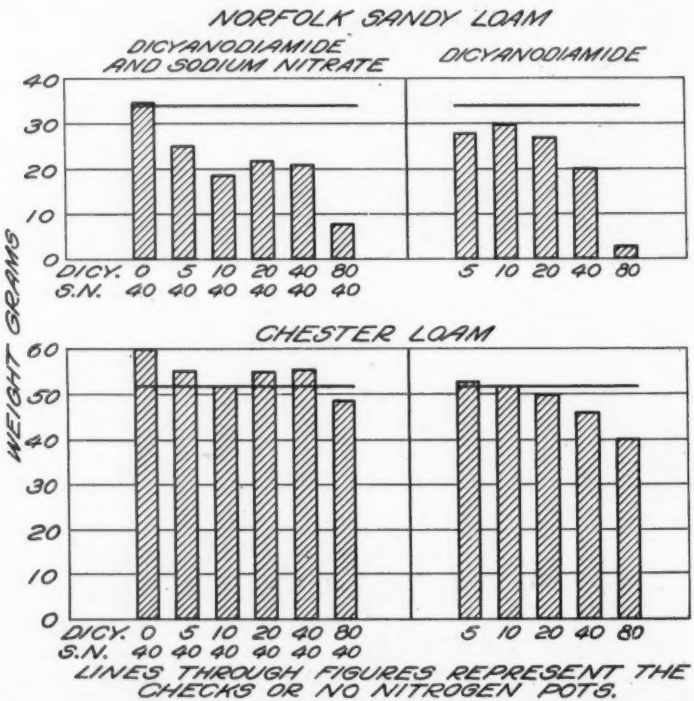


FIG. 2.—Diagram showing the yield of cowpeas from pots receiving various amounts of dicyanodiamide and sodium nitrate

The results with the Chester loam agree very closely with those in Table V in showing a fair increase in growth with sodium nitrate. Furthermore, the dicyanodiamide injury was slightly less in the presence of nitrate nitrogen, 10 pounds of the ammonia equivalent being sufficient to more than counteract 40 pounds of ammonia as dicyanodiamide. The leaf yellowing and other visible signs of the dicyanodiamide poisoning were not lessened in the presence of nitrate nitrogen.

marked difference between the toxicity of dicyanodiamide for wheat and for cowpeas was especially noticeable. The injury to wheat was only slight, even at the high concentration of 40 pounds of the ammonia equivalent per acre. For cowpeas, 5 pounds of ammonia as dicyanodiamide was decidedly toxic.

Where used on wheat, dicyanodiamide at the rate of 40 pounds of ammonia per acre produced some tip burning and decreased the green weights of the

plants 17 per cent on the Norfolk sandy loam and 13 per cent on the Chester loam. In the presence of sodium nitrate dicyanodiamide produced the tip burning but did not markedly decrease the value of sodium nitrate. This indicates that dicyanodiamide is not a marked direct poison for wheat but is merely unavailable as a plant food and probably prevents the proper utilization of the soil nitrogen.

Experiments to determine the residual effects of dicyanodiamide showed that the material remained in the pots and produced a marked decrease in the second crop (cowpeas) on the sandy soil whether previously used with or without sodium nitrate. On the loam soil the residual effects were almost negligible.

Additional experiments where cowpeas were grown in the presence of fresh

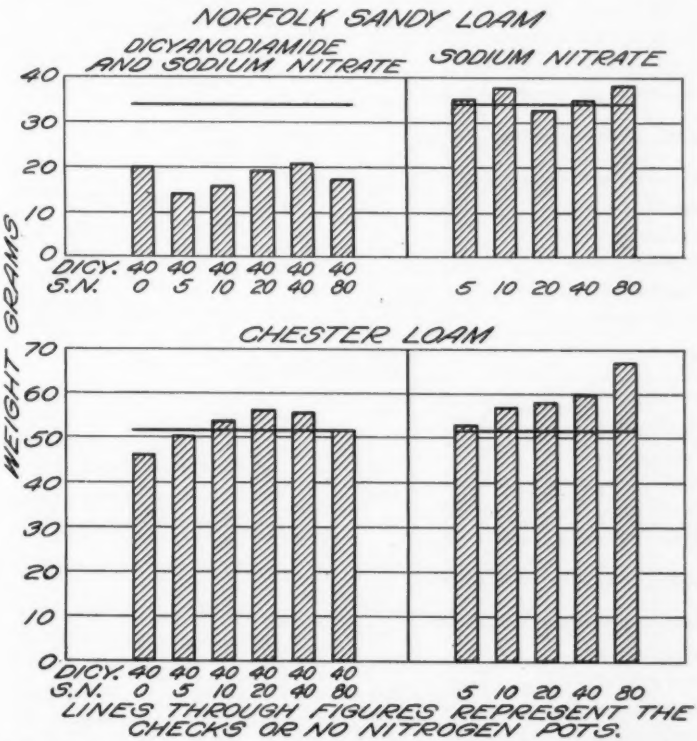


FIG. 3.—Diagram showing the yield of cowpeas from pots receiving various amounts of dicyanodiamide and sodium nitrate

In the presence of 40 pounds of ammonia as dicyanodiamide, sodium nitrate equivalent to 5 pounds of ammonia on the lighter soil and 10 pounds on the heavier was sufficient to counteract the decrease in the green weights of the wheat plants produced by the dicyanodiamide. Where sodium nitrate and dicyanodiamide were used in varying proportions, the green weights depended primarily upon the nitrate supply, the dicyanodiamide exerting only a slight injury and its nitrogen being unavailable.

applications of dicyanodiamide showed that this plant is injured even by small applications of the material (5 pounds of ammonia per acre). Sodium nitrate usually did not counteract the injury and even increased it in several instances. Practically all plants grown in the presence or absence of nitrate nitrogen showed a yellowing of the lower leaves and a slight mosaic appearance of the others. With the larger applications on the sandy soil germination was affected and the plants were nearly dead when the experiment was terminated.

TABLE V.—*Green weights of cowpeas from pots receiving various quantities of dicyanodiamide with and without sodium nitrate*

NORFOLK SANDY LOAM

Source of nitrogen	Fertilizer ratio NH_3–P_2O_5–K_2O	Green weight	Average	Variation from check	Nodule formation
		Grams.			
No nitrogen	0-8-4	32.6 35.5	34.1	----------	Abundant. Do.
Sodium nitrate 40	4-8-4	33.9 35.5	34.7	0.6	Do. Do.
Sodium nitrate 40, dicyanodiamide 5	4.5-8-4	24.9 25.1	25.0	−9.1	Many. Abundant.
Sodium nitrate 40, dicyanodiamide 10	5-8-4	17.1 19.7	18.4	−15.7	Many. Abundant.
Sodium nitrate 40, dicyanodiamide 20	6-8-4	17.2 25.9	21.6	−12.5	Many. Do.
Sodium nitrate 40, dicyanodiamide 40	8-8-4	20.9 25.8 20.3 16.6	20.9	−13.2	Do. Do. Do. Do.
Sodium nitrate 40, dicyanodiamide 80	12-8-4	4.3 9.9	7.1	−27.0	None. Many.
Dicyanodiamide 5	.5-8-4	27.4 27.9	27.7	−6.4	Do. Do.
Dicyanodiamide 10	1-8-4	33.3 26.3	29.8	−4.3	Abundant. Many.
Dicyanodiamide 20	2-8-4	23.9 29.8	26.9	−7.2	Abundant. Do.
Dicyanodiamide 40	4-8-4	24.1 15.7	19.9	−14.2	Do. Many.
Dicyanodiamide 80	8-8-4	1.4 3.8	2.6	−31.5	None. Few.

CHESTER LOAM

No nitrogen	0-8-4	50.6 52.4	51.5	----------	Few. Do.
Sodium nitrate 40	4-8-4	58.6 61.6	60.1	8.6	Do. None.
Sodium nitrate 40, dicyanodiamide 5	4.5-8-4	56.4 53.6	55.0	3.5	Do. Few.
Sodium nitrate 40, dicyanodiamide 10	5-8-4	54.7 48.1	51.4	−.1	None Few.
Sodium nitrate 40, dicyanodiamide 20	6-8-4	57.3 52.3	54.8	3.3	Do. None.
Sodium nitrate 40, dicyanodiamide 40	8-8-4	57.7 53.8 53.7 56.1	55.3	3.8	Few. None. Do. Few.
Sodium nitrate 40, dicyanodiamide 80	12-8-4	51.9 45.3	48.6	−2.9	Do. Do.
Dicyanodiamide 5	.5-8-4	51.6 54.1	52.9	1.4	Do. None.
Dicyanodiamide 10	1-8-4	54.1 49.7	51.9	.4	Few. Do.
Dicyanodiamide 20	2-8-4	47.1 52.8	50.0	−1.5	Do. Do.
Dicyanodiamide 40	4-8-4	42.9 49.0	46.0	−5.5	Do. None.
Dicyanodiamide 80	8-8-4	37.5 43.0	40.3	−11.2	Few. Do.

The difference between the extent of the injury of dicyanodiamide on wheat and cowpeas suggests an explanation for some of the widely varying results reported in the literature. For one crop the material may be very toxic, even in small amounts, while for other plants it is nearly inert or indirectly toxic because of its prevention of nitrification. This emphasizes the desirability of further work with other crops. However, dicyanodiamide was somewhat toxic even for the more resistant crop, wheat, and for this reason the material should not be present in appreciable quantities in fertilizers.

TABLE VI.—*Green weights of cowpeas from pots receiving various amounts of sodium nitrate with and without dicyanodiamide*

NORFOLK SANDY LOAN

Source of nitrogen	Fertilizer ratio NH_3-P_2O_5-K_2O	Green weight	Average	Variation from check	Nodule formation
		Grams			
No nitrogen	0-8-4	32.6	34.1	----------	Abundant.
		35.5			Do.
Dicyanodiamide 40	4-8-4	24.1	19.9	−14.2	Do.
		15.7			Many.
Dicyanodiamide 40, sodium nitrate 5	4.5-8-4	13.1	13.8	−20.3	Do.
		14.5			Do.
Dicyanodiamide 40, sodium nitrate 10	5-8-4	15.1	15.5	−18.6	Do.
		15.9			Do.
Dicyanodiamide 40, sodium nitrate 20	6-8-4	16.8	19.0	−15.1	Abundant.
		21.1			Many.
		20.3			Do.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	16.6			Do.
		20.9	20.9	−13.2	Do.
		25.8			Do.
Dicyanodiamide 40, sodium nitrate 80	12-8-4	15.5	17.4	−16.7	Few.
		19.2			Many.
Sodium nitrate 5	.5-8-4	38.5	35.0	.9	Do.
		31.5			Do.
Sodium nitrate 10	1-8-4	36.5	37.7	3.6	Do.
		38.9			Do.
Sodium nitrate 20	2-8-4	31.9	32.8	−1.3	Do.
		33.7			Do.
Sodium nitrate 40	4-8-4	33.9	34.7	.6	Abundant.
		35.5			Do.
Sodium nitrate 80	8-8-4	37.6	38.3	4.2	Many.
		38.9			Do.

CHESTER LOAM

No nitrogen	0-8-4	50.6	51.5	----------	Few.
		52.4			Do.
Dicyanodiamide 40	4-8-4	42.9	46.0	−5.5	Do.
		49.0			None.
Dicyanodiamide 40, sodium nitrate 5	4.5-8-4	51.8	50.3	−1.2	Few.
		48.8			Do.
Dicyanodiamide 40, sodium nitrate 10	5-8-4	55.1	53.1	1.6	None.
		51.0			Few.
Dicyanodiamide 40, sodium nitrate 20	6-8-4	54.5	55.9	4.4	Do.
		57.3			None.
		53.7			Do.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	56.1	55.3	3.8	Few.
		57.7			Do.
		53.8			None.
Dicyanodiamide 40, sodium nitrate 80	12-8-4	56.0	51.5	.0	Do.
		46.9			Do.
Sodium nitrate 5	.5-8-4	52.0	52.2	.7	Few.
		52.3			Do.
Sodium nitrate 10	1-8-4	57.7	56.3	4.8	Do.
		54.9			Do.
Sodium nitrate 20	2-8-4	60.8	57.3	5.8	None.
		53.8			Few.
Sodium nitrate 40	4-8-4	58.6	60.1	8.6	Do.
		61.6			None.
Sodium nitrate 80	8-8-4	60.9	66.2	14.7	Few.
		65.4			Do.

STUDIES ON THE SINGLE-INJECTION METHOD OF VACCINATION AS A PROPHYLACTIC AGAINST RABIES IN DOGS [1]

By HARRY W. SCHOENING

Associate Veterinarian, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

Immunization against rabies has been practiced for many years in both human and veterinary medicine. Its use has been confined almost exclusively to cases of exposure to rabies infection, and by means of the Pasteur and Högyes treatments or their modifications the mortality from the disease has been greatly reduced. These methods, while efficient, are not applicable as a practicable means of controlling rabies in dogs—the principal source of propagation of the disease—inasmuch as a number of injections are required with the consequent entailment of considerable expense.

Owing to the increase of rabies in Japan, Umeno and Doi [2] sought by means of vaccination a method for the control of the disease in dogs. After experimental work they concluded that it was possible to immunize dogs against rabies by means of one subcutaneous injection of glycerinated fixed virus which had been attenuated by the addition of phenol. This method was then put into practical use and large numbers of pet dogs in certain districts were given one dose of vaccine.

Umeno and Doi state:

> By summing up the statistics obtained in Kanagawa and Tokyo Prefectures we obtain the following figures:
>
> There were 31,307 vaccinated dogs, of which number there was only one case of vaccination loss and one in which the vaccination was not to be regarded effective enough. There appeared quite a number of rabid dogs among the nonvaccinated dogs. Thus, we see the great importance of the vaccination of dogs in order to prevent the spread of rabies among dogs.

Eichhorn and Lyon [3] and Reichel and Schneider [4] report experimental work with this method in the United States.

The striking results of the practical application of the single-injection vaccine, as reported by Umeno and Doi, in Japan, together with the experimental results reported by Eichhorn and Lyon in the United States in 1922, led to the receipt by the Bureau of Animal Industry, United States Department of Agriculture, of numerous letters from veterinarians, public health officials, and dog owners requesting information as to the efficiency of this method of immunization and its probable use in the control and eventual eradication of rabies from communities.

As rabies appears to be on the increase, particularly in certain sections of the country, and as the control of the disease is an important problem, not only from an economic point of view but also from a public-health standpoint, any method looking to the control of the disease in dogs deserves thorough consideration and study. Experimental work on this subject was therefore undertaken.

PREPARATION OF THE VACCINE

According to the method of Umeno and Doi, the vaccine is prepared as follows:

The brain and spinal cord of a rabbit dead of fixed rabies virus infection are removed and thoroughly ground in a mortar. To the ground-up mass four times its volume of phenolized glycerin water is added. The glycerin water consists of 60 parts of glycerin and 40 parts of water which contains 1.25 per cent phenol, making the glycerinated water contain 0.5 per cent phenol. After being thoroughly mixed, the emulsion is allowed to stand at room temperature of 18° to 22° C. for two weeks, or in the ice box for 30 days, after which time it is ready for use.

[1] Received for publication June 16, 1924; issued May, 1925.

[2] UMENO, S., and DOI, Y. A STUDY ON THE ANTI-RABIC INOCULATION OF DOGS AND THE RESULTS OF ITS PRACTICAL APPLICATION. Kitasato Arch. Exp. Med. 4: 89-108, 1921.

[3] EICHHORN, A., and LYON, B. M. PROPHYLACTIC VACCINATION OF DOGS AGAINST RABIES. Jour. Amer. Vet. Med. Assoc. (n. s. 14) 61: 38-42. 1922.

[4] REICHEL, J., and SCHNEIDER, J. E. RABIES VACCINE CANINE. SINGLE DOSE TREATMENT. Jour. Amer. Vet. Med. Assoc. (n. s. 16) 63: 83-84. 1923.

This technic is followed more or less closely by the majority of the commercial houses manufacturing this product. In several instances, however, the fixed virus is killed by phenol, the resultant vaccine being a killed rather than an attenuated fixed virus.

SOURCE AND DOSAGE OF VACCINES

The vaccines used in the following experiments consisted of several lots prepared in the laboratory of the Bureau of Animal Industry, and of lots obtained from commercial houses manufacturing this product. The vaccines prepared in this laboratory were made according to the method of Umeno and Doi, with the attenuation taking place at room temperature for two weeks. The dosage was 3 c. c. for dogs under 20 pounds, 5 c. c. for dogs between 20 and 40 pounds, and 10 c. c. for dogs over 40 pounds.

The commercial vaccines were received in original sealed containers and were kept in the ice box until used. The dates on which the vaccines were used were well within the expiration dates marked on the outside containers, and the dose given was according to the manufacturers' directions.

EXPERIMENTAL DATA

METHOD OF INJECTION

The loose skin back of the shoulder or elbow of the dog was shaved, the skin disinfected with 3 per cent phenol, dried, and painted with 3.5 per cent tincture of iodine. The proper amount of vaccine was then slowly injected subcutaneously in two or three places.

After varying intervals of time the vaccinated dogs, together with a number of control dogs, received an intraocular injection of rabies street virus.

EFFECT OF THE INJECTION OF VACCINE

The dogs were not inconvenienced by the injection of the vaccine. They remained lively and ate heartily at all times. In one case, however, the injection of the vaccine resulted in a death from rabies. Dog 440 was injected with 5 c. c. of Bureau of Animal Industry vaccine No. 1 on May 25, 1922. On September 13, 1922, the dog showed signs of paralysis and on September 14 was stretched out on its side, completely paralyzed. It continued in this condition through September 15, and was found dead on the morning of September 16. Microscopic examination of smears from the hippocampus revealed the presence of a few small Negri bodies.

Three rabbits inoculated subdurally with brain material from dog 440 were completely paralyzed on the seventh day after inoculation and died two to three days later. Microscopic examination of the brains of these three rabbits revealed the presence of a few small Negri bodies of the type usually seen in fixed virus.

Reinoculation of rabbits with the brain of one of these rabbits was followed by typical rabies paralysis in the inoculated animals on the seventh day.

Several more passages of this virus were made in rabbits, and in every instance complete paralysis occurred on the seventh day.

These inoculation results clearly indicate that dog 440 died of rabies fixed-virus infection. Three other dogs received the same amount of this vaccine and on the same day as dog 440, but remained perfectly healthy. This was the only case of death as a result of the injection of the vaccine.

METHOD OF EXPOSURE TO STREET VIRUS

One of the difficulties encountered in experimental work on rabies immunization is the question of the mode of exposure and the amount of street virus to be injected. It is extremely difficult or impossible to standardize street virus, owing to the variable action of the same amount of virus on different individuals. Probably with no other disease does individual resistance play so important a part as in rabies. What is highly virulent for one dog may not be sufficient to cause the development of the disease in another.

For an experiment to be successful the exposure to street virus should be sufficiently virulent to cause the development of the disease, if not in all, at least in the great majority of the control dogs. On the other hand, the virus should not be so virulent as to be simply overwhelming.

The intraocular injection is a severe test, probably much more so than an animal would encounter naturally. However, as an experimental means of exposure it is probably better than any other method. Umeno and Doi, Eichhorn and Lyon, and Reichel and Schneider report the use of this method in experimental work, and it is the method used in the following experiments.

EXPERIMENT NO. 1

On May 25, 1922, 7 dogs each received one injection of vaccine. On July 18, 11

additional dogs each received one injection, making a total of 18 vaccinated dogs. Nine of these were given a vaccine made in this laboratory (designated B. A. I.) and 9 were vaccinated with vaccines prepared by three commercial firms (designated respectively A, B, and C.). On September 12, 1922, the 18 vaccinated dogs together with 8 control dogs received an injection of 0.1 c. c. of rabies street virus in the anterior chamber of the left eye. The virus consisted of a 1:10 dilution in salt solution of the hippocampus and medulla of a dog dead of rabies.

The rabies street virus used in experiment No. 1 was obtained from a dog which developed the disease spontaneously at the Bureau of Animal Industry Experiment Station, Bethesda, Md. This dog, No. 474, was purchased from the District of Columbia pound on August 22, 1922, and was being held for use as a control or check dog in a rabies experiment. On September 10 the animal developed clinical symptoms of furious rabies and was found dead September 11. The diagnosis of rabies was confirmed by microscopic examination and subsequently by rabbit inoculations. On September 12 a 1:10 dilution of the hippocampus and medulla of this dog was made in salt solution and used as the exposure virus in experiment No. 1. This virus was designated Bureau of Animal Industry No. 474.

The data of experiment No. 1 are given in Table I.

TABLE I.—*Data of experiment No. 1*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
1	A	5	May 25, 1922	Sept. 12, 1922	Dead Nov. 2, 1922; rabies.	Nov. 2, 1922	Dead, Nov. 16, 1922; rabies.
2	A	5	do	do	Dead, Sept. 30, 1922; rabies.	Sept. 30, 1922	Dead, Oct. 14, 1922; rabies.
3	B	5	do	do	Dead, Sept. 29, 1922; rabies.	do	Dead Oct. 15, 1922; rabies.
4	B. A. I.	5	do	do	Dead, Oct. 2, 1922; rabies.	Oct. 3, 1922	Do.
5	B. A. I.	5	do	do	Alive, Mar. 7, 1923.		
6	B. A. I.	5	do	do	Dead, Sept. 29, 1922; rabies.	Sept. 30, 1922	Do.
7	B	5	do	do	Alive, Mar. 7, 1923.		
8	A	5	July 18, 1922	do	Dead, Sept. 24, 1922; rabies.	Sept. 27, 1922	Dead, Oct. 17, 1922; rabies.
9	B. A. I.	3	do	do	Dead, Sept. 26, 1922; rabies.	do	Dead, Oct. 9, 1922; rabies.
10	B. A. I.	5	do	do	Alive, Mar. 7, 1923.		
11	B. A. I.	5	do	do	Dead, Sept. 28, 1922; rabies.	Sept. 30, 1922	Dead, Oct. 15, 1922; rabies.
12	B. A. I.	3	do	do	Dead, Sept. 25, 1922; rabies.	Sept. 28, 1922	Dead, Oct. 9, 1922; rabies.
13	C	5	do	do	Dead, Sept. 24, 1922; rabies.	Sept. 27, 1922	Dead, Oct. 11, 1922; rabies.
14	B. A. I.	5	do	do	do	Sept. 26, 1922	Dead, Oct. 9, 1922; rabies.
15	B. A. I.	5	do	do	do	Dec. 7, 1922	Dead, Dec. 21, 1922; rabies.
16	B	5	do	do	do	Sept. 26, 1922	Dead, Oct. 6, 1922; rabies.
17	B	5	do	do	Dead, Sept. 25, 1922; rabies.	Sept. 28, 1922	Dead, Oct. 17, 1922; rabies.
18	B	3	do	do	Dead, Sept. 28, 1922; rabies.	do	Dead, Oct. 11, 1922; rabies.
19	Control dog.			do	Dead, Oct. 24, 1922; enteritis.	Oct. 24, 1922	Alive, Dec. 24, 1922.
20	do			do	Alive, Jan. 16, 1923.		
21	do			do	do		
22	do			do	do		
23	do			do	do		
24	do			do	do		
25	do			do	do		
26	do			do	Dead, Oct. 9, 1922; rabies.		

[a] Vaccines A, B, and C were from three different commercial houses manufacturing this product. Vaccine B. A. I. was prepared in the laboratory of the Bureau of Animal Industry.

[b] The virus consisted of a 1 : 10 dilution in salt solution of the hippocampus and medulla of dog 474, dead of rabies; 0.1 c. c. of this material was injected into the anterior chamber of the left eye.

[c] The diagnosis of rabies was made by microscopic examination, and in the case of vaccinated dogs by rabbit inoculations. The surviving control dogs were destroyed Jan. 16, 1923, and the surviving vaccinated dogs were destroyed Mar. 7, 1923.

Table I may be summarized as follows: Eighteen dogs received one injection of vaccine, 7 on May 25 and 11 on July 18. On September 12 the 18 vaccinated dogs together with 8 control dogs received an intraocular injection of street virus. Of the 18 vaccinated dogs, 15 died of rabies. Of the 8 control dogs 1 died of rabies and 1 of enteritis.

In this and the following experiments the diagnosis of rabies in vaccinated animals was always confirmed by microscopic examination and rabbit inoculations.

The irregular outcome of this experiment is rather difficult of interpretation. The first thought that came to mind was the possibility of the vaccine sensitizing the animals, so that they succumbed to infection more quickly than nonvaccinated dogs. Subsequent experiments have not proved this to be the case, however, and the following is probably the cause of the irregularity of the result: The 18 vaccinated dogs were housed in one barn, and these were the first to be exposed to the street virus. The 8 control dogs were housed in a second barn about one-fourth of a mile from the first. After the dogs in the first barn had been exposed the virus was carried in a small beaker to the second barn, where the control dogs were then injected. Exposure to sunlight or to some unknown factor probably caused an alteration of the virus between the first and second barns. The injections in both groups of animals were made by the writer, and the material was stirred well before each injection.

Although the result of experiment No. 1 was irregular, it showed conclusively that the single injection of a vaccine did not produce sufficient immunity to protect dogs against an intraocular injection of the street virus used.

EXPERIMENT NO. 2

It was decided to repeat the previous experiment. Three dogs were vaccinated on July 18 and three on October 11, 1922. On October 26 these six dogs, together with three control dogs, received an intraocular injection of rabies street virus, 0.1 c. c. of a 1:10 dilution of the hippocampus and medulla of dog 26, dead of rabies (B. A. I. virus 474). The results are given in Table II.

Summarizing Table II it appears that five of the six vaccinated dogs died of rabies. The death of the sixth could not be attributed to rabies. All three controls died of rabies.

The diagnosis of rabies in the vaccinated animals was confirmed by microscopic examination and rabbit inoculations. In the control dogs the diagnosis was made clinically and by the demonstration of Negri bodies microscopically.

The incubation period of the disease was about the same in the vaccinated dogs as in the controls, showing that

TABLE II.—*Data of experiment No. 2*

Dog No.	Vaccine[a]	Amount injected	Date vaccine was injected	Date exposed to virus[b]	Result[c]	Date of rabbit inoculation	Result
		C. c.					
27	B	5	July 18, 1922	Oct. 26, 1922	Dead, Dec. 23, 1922; rabies.	Dec. 27, 1922	Dead, Jan. 25, 1923; rabies.
28	C	3	do	do	Dead, Nov. 21, 1922; rabies.	Nov. 23, 1922	Dead, Dec. 7, 1923; rabies.
29	A	5	do	do	Dead, Nov. 15, 1922; rabies.	Nov. 16, 1922	Dead, Dec. 26, 1923; rabies.
30	B. A. I.	5	Oct. 11, 1922	do	Dead, Nov. 24, 1922; rabies.	Nov. 25, 1922	Dead, Dec. 12, 1923; rabies.
31	do	3	do	do	Dead, Dec. 8, 1922; rabies.	Dec. 12, 1922	Dead, Dec. 26, 1923; rabies.
32	do	5	do	do	Died suddenly, Dec. 14, 1922; not rabies.	Dec. 15, 1922	Alive, Feb. 15, 1923.
33	Control dog.			do	Dead, Nov. 24, 1922; rabies.		
34	do			do	Dead, Nov. 10, 1922; rabies.		
35	do			do	Dead, Dec. 14, 1922; rabies.		

[a] Vaccines A, B, and C were from three different commercial houses manufacturing this product. Vaccine B. A. I. was prepared in the laboratory of the Bureau of Animal Industry.

[b] The virus consisted of a 1:10 dilution in salt solution of the medulla and hippocampus of dog 26 (B. A. I 474); 0.1 c. c. of this material was injected into the anterior chamber of the left eye.

[c] Diagnosis of rabies was made by microscopic examination and, in the case of vaccinated dogs, by rabbit inoculations.

the vaccination had no appreciable effect on the incubation period of the disease. If the period of incubation in the control animals can be used as an index of the virulence of the virus, it appears that the animals were not overwhelmed with virus.

This experiment confirmed the conclusions drawn from experiment No. 1, namely, that a single injection of vaccine failed to produce sufficient immunity to protect dogs against an intraocular injection of street virus B. A. I. 474.

The results of the two foregoing experiments were in contradiction to the results obtained by Eichhorn and Lyon, who protected 100 per cent of their animals against an intraocular injection of street virus.

In comparing experimental data of Eichhorn and Lyon with the preceding experiments, it was observed that the only practical differences between the experiments were the nature of the exposure virus used and the personal factor of technic. Leaving the personal factor aside, the only difference remaining was the use of a different exposure virus. It was considered advisable at this time to inquire into the source of the fixed virus used by the various commercial houses manufacturing the single-injection canine rabies vaccine.

As a result of this inquiry it was learned with considerable surprise that the virus used by all the commercial houses apparently had a common origin, the Pasteur Institute of Paris, France. The canine vaccines manufactured in this country, therefore, are prepared with a European strain of fixed virus. As a number of the commercial houses manufacturing canine rabies vaccine also prepare rabies vaccine for human use, it is probable that the fixed virus used for this product also had the same source.

There was a possibility, it was believed, that there might be different strains of rabies street virus against which, or some of which, protection could not be obtained by immunization with the strain of fixed virus used as a vaccine. To determine this point was the object of experiment No. 3.

EXPERIMENT NO. 3

Eighteen dogs were given one injection of vaccine. They were then divided into three groups of six each, and to each group were added four normal dogs to be used as controls.

Each group of 10 dogs (6 vaccinated and 4 controls) was exposed to an intraocular injection of a street virus of different origin.

Virus No. 1 had the following history: Pennsylvania dog to man, to rabbit, to rabbit, to dog; 0.1 c. c. of a 1:2 dilution of the brain of the dog was used for exposure.

Virus No. 2 was the B. A. I. 474 strain, a 1 : 10 dilution of the medulla of dog No. 35, dead December 14, and in glycerin since that time.

Virus No. 3 consisted of small pieces of rabid brain material of a New York dog, cat, and calf. This material, however, had been in glycerin for a considerable period of time.

The results of this experiment are given in Table III.

Table III may be summarized as follows: In lot No. 1, exposed to virus No. 1, one vaccinated dog died of rabies and five survived. Of the four control dogs, all died of rabies. Sixteen and two-thirds per cent of the vaccinated dogs died, as against 100 per cent of the control dogs, indicating that considerable protection was afforded by vaccination against this virus.

In lot No. 2, exposed to B. A. I. 474 virus, four vaccinated dogs died of rabies and two survived. Of the four control dogs, three died of rabies and one survived. Sixty-six and two-thirds per cent of the vaccinated dogs died, as against 75 per cent of the control dogs. This confirms the previous experiment in which little or no protection was afforded by vaccination against this virus.

Attention is called to dog 47 in this lot, which showed symptoms of furious rabies March 5 and was found dead on the morning of March 6. Extracellular Negri bodies of average size were found. Rabbits inoculated with brain material of this dog were completely paralyzed on the seventh day. Four passages of this virus through rabbits resulted in each instance in the development of complete paralysis on the seventh day. Negri bodies in these rabbits were scarce, but of very fair size. These results indicate that the virus in the brain of dog 47 had the properties of a fixed virus. Whether the exposure virus became fixed for the rabbit after passage through this dog, or whether the animal succumbed to fixed-virus infection from the vaccine which became exalted as a result of the injection of the street virus, are questions difficult to answer.

In lot No. 3, exposed to virus No. 3, one vaccinated dog died of rabies after an incubation period of almost four

TABLE III.—*Data of experiment No. 3*

LOT NO. 1

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
36	B	5	Nov. 18, 1922	Feb. 1, 1923	Alive, Nov. 1, 1923.		
37	C	5	Dec. 13, 1922	do	do		
38	B.A.I.	5	Nov. 28, 1922	do	do		
39	A	5	Nov. 21, 1922	do	do		
40	do	5	Nov. 28, 1922	do	Dead, Feb. 16, 1923; rabies.	Feb. 17, 1923	Dead, Feb. 28, 1923; rabies.
41	D	5	Nov. 21, 1922	do	Alive, Nov. 1, 1923.		
42	Control dog			do	Dead, Apr. 5, 1923; rabies.	Apr. 7, 1923	Dead, Apr. 25, 1923; rabies..
43	do			do	Dead, Mar. 17, 1923; rabies.		
44	do			do	Dead, June 7, 1924.	June 9, 1924	Dead, June 25, 1924; rabies.
45	do			do	Dead, Feb. 22, 1923; rabies.		

LOT NO. 2

46	B	5	Nov. 21, 1922	Feb. 20, 1923	Dead, Mar. 10, 1923; rabies.	Mar. 24 1923	Dead, Apr. 4, 1923; rabies.
47	C	5	Dec. 13, 1922	do	Dead, Mar. 6, 1923; rabies.	Mar. 8, 1923	Dead, Mar. 16, 1923; rabies.
48	B. A. I.	5	Nov. 28, 1922	do	Killed, May 10, 1923; by dog 39.		
49	do	5	do	do	Dead, Apr. 2, 1923; rabies.	Apr. 3, 1923	Dead, Apr. 16 1923; rabies.
50	A	5	Nov. 21, 1922	do	Alive, Nov. 1, 1923.		
51	D	5	do	do	Dead, Apr. 9, 1923; rabies.	Apr. 16, 1923	Dead, Apr. 27, 1923; rabies.
52	Control dog.			do	Alive, Nov. 1, 1923.		
53	do			do	Dead, Mar. 6, 1923; rabies.		
54	do			do	Dead, Mar. 5, 1923; rabies.		
55	do			do	Dead, Mar. 6, 1923; rabies.		

LOT NO. 3

56	B	5	Nov. 28, 1922	Feb. 21, 1923	Alive, Nov. 1, 1923.		
57	do	5	Dec. 13, 1922	do	do		
58	B. A. I.	5	Nov. 28, 1922	do	do		
59	A	5	Jan. 13, 1922	do	do		
60	D	5	Nov 28, 1922	do	do		
61	do	5	Dec. 13, 1922	do	Dead, June 16, 1923; rabies.	June 18, 1923	Dead, June 29, 1923; rabies.
62	Control dog.			do	Killed, June 19, 1923; by dog 56.		
63	do			do	Alive, Nov. 1, 1923.		
64	do			do	do		
65	do			do	do		

[a] Vaccines A, B, C, and D were from four different commercial houses manufacturing this product. B. A. I. vaccine was prepared in the laboratory of the Bureau of Animal Industry.

[b] Lot No. 1 was exposed to virus No. 1, lot No. 2 to virus No. 2, and lot No. 3 was exposed to virus No. 3. In lots Nos. 1 and 3 the virus consisted of a 1:10 dilution of the hippocampus and medulla of a dog dead of rabies of a different source. Lot No. 2 consisted of a 1:2 dilution of the medulla and hippocampus. One-tenth of a cubic centimeter was injected into the anterior chamber of the eye.

[c] Diagnosis of rabies was made by microscopic examination, and in the case of vaccinated dogs and control dogs 42 and 44 also by rabbit inoculations.

months. None of the remaining vaccinated dogs nor any of the control dogs developed the disease. The small pieces of brain material composing this virus and the length of time in glycerin evidently led to its deterioration. It is significant to note, however, that the only animal to succumb in this lot was a vaccinated one. This experiment appears to indicate that a certain amount of immunity can be produced by a single injection of vaccine against one strain of virus, but practically none against another strain.

EXPERIMENT NO. 4

The fourth experiment was a repetition of the previous experiments, except that a still different street virus was used for exposure.

The source of the street virus was Washington, D. C.; dog to rabbit, to dog 630. A 1:10 dilution of the hippocampus and medulla of dog 630 was used for exposure virus.

The results are given in Table IV.

Table IV may be summarized as follows: Ten dogs were given a single injection of vaccine, and later, together with 6 control dogs, were given an intraocular injection of street virus from dog 630. Of the 10 vaccinated dogs 3, or 30 per cent, died of rabies, while of the 6 control dogs all succumbed to the disease.

In this experiment distinct protection was afforded by a single injection of vaccine. All the control animals succumbed to the disease with a short incubation period, which probably indicates a high degree of virulence for the exposure virus and a severe test on the efficiency of the method of vaccination.

EXPERIMENT NO. 5

The fifth experiment had for its object the exposure of vaccinated and nonvaccinated dogs to the bites of a rabid animal.

Dogs 56, 57, 58, and 59 were vaccinated animals, and dogs 63, 64, and 65 were controls used in experiment No. 3, lot No. 3, which had failed to develop rabies after intraocular exposure. Dogs 82 and 83 were normal dogs used as controls.

The dog to which the abovementioned animals were exposed was the one which after death furnished the virus for exposure in experiment No. 4. The source of the virus was

TABLE IV.—*Data of experiment No. 4*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
66	A	5	June 14, 1923	Nov. 20, 1923	Alive, April 20, 1924.		
67	B	5	do	do	do		
68	A	5	do	do	do		
69	C	5	Aug. 4, 1923	do	do		
70	C	5	do	do	do		
71	D	5	do	do	do		
72	B	5	Sept. 5, 1923	do	Dead, Jan. 17, 1924; rabies.	Feb. 4, 1924	Dead, Feb. 19, 1924; rabies.
73	B	5	do	do	Dead, Dec. 26, 1923; rabies.	Dec. 27, 1923	Dead, Jan. 11, 1924; rabies.
74	D	5	do	do	Dead, Dec. 10, 1923; rabies.	Dec. 11, 1923	Dead, Dec. 27, 1923; rabies.
75	D	5	do	do	Alive, Apr. 20, 1924.		
76	Control dog.			do	Dead, Dec. 11, 1923; rabies.		
77	do			do	Dead, Dec. 8, 1923; rabies.		
78	do			do	Dead, Dec. 12, 1923; rabies.		
79	do			do	Dead, Dec. 11, 1923; rabies.		
80	do			do	Dead, Dec. 10, 1923; rabies.		
81	do			do	Dead, Dec. 7, rabies.		

[a] Vaccines A, B, C, and D were from four different commercial houses manufacturing this product.
[b] The virus consisted of a 1:10 dilution of the medulla and hippocampus of dog 630 dead of rabies; 0.1 c. c. of this material was injected into the anterior chamber of the left eye.
[c] Diagnosis of rabies was made by microscopic examination, and in vaccinated dogs was also confirmed by rabbit inoculations.

Washington, D. C.; dog to rabbit to dog 630. Dog 630, the animal used, was inoculated intraocularly on October 24, 1923. On November 12, 1923, the animal presented clinical sumptoms of rabies.

Each dog in the experiment was placed in the same cage with dog 630. In each instance the animals were attacked and bitten by the rabid dog. Each animal was left in the cage until it was definitely seen that wounds were inflicted. The effort was made to give all the dogs the same degree of exposure. The results of the test are given in Table V.

Against a still different strain of street virus (B. A. I. 474), however, practically no protection was afforded to vaccinated animals exposed by intraocular injection. Of 30 vaccinated dogs exposed to this virus, 6 survived.

A study of the vaccines indicates that the source, age, etc., had no bearing on the outcome of the various experiments. All the vaccines used in these experiments were prepared from the same strain of fixed virus, which had its origin in the Pasteur Institute of Paris, France. The results of the experiments herein reported indicate that this strain of fixed virus, when used as a

TABLE V.—*Data of experiment No. 5*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]
		C. c.			
56	B	5	Nov. 28, 1922	Nov. 12, 1923	Alive Apr. 12, 1924.
57	B	5	Dec. 13, 1922	do	Do.
58	B. A. I.	5	Nov. 28, 1922	do	Do.
59	A	5	Jan. 13, 1922	do	Do.
63				do	Dead Dec. 3, 1923; rabies.
64				do	Alive Apr. 12, 1924.
65				do	Do.
82				do	Do.
83				do	Dead Dec. 3, 1923; rabies.

[a] Vaccines A and B were from two different commercial houses manufacturing this product. Vaccine B. A. I. was prepared in the laboratory of the Bureau of Animal Industry.
[b] These animals were exposed to the bites of a rabid dog, No. 630.
[c] Diagnosis of rabies made clinically and by microscopic examination.

Experiment No. 5 may be summarized as follows: Four vaccinated dogs and five control dogs were exposed to the bites of a rabid animal; two of the five controls succumbed to rabies. None of the vaccinated animals developed the disease.

The number of animals exposed to rabies and not treated in which infection developed has been estimated by various investigators to be between 16 and 60 per cent. In this experiment 40 per cent of the nonvaccinated animals bitten developed the disease. In view of the fact that none of the vaccinated animals developed the disease, it can be assumed that the vaccine afforded protection in this experiment.

DISCUSSION

From the foregoing experiments it appears that the efficacy of the single injection canine rabies vaccine as a prophylactic depends on the street virus to which the vaccinated animal is exposed. Against intraocular injections of two different strains of street virus, distinct protection was afforded by the vaccine, 12 out of the 16 vaccinated dogs being protected.

vaccine, protects against certain strains of street virus, but that one strain of street virus was encountered against which no protection could be obtained.

The question as to whether these results are due to a difference in virulence of the virus used and not to a distinct difference of strain can be answered, it is believed, by a comparison of experiments Nos. 2 and 4.

In experiment No. 2, using the strain of virus B. A. I. 474, the three control dogs died of rabies in 15, 29, and 49 days. Assuming the incubation period of the disease to be an index to the virulence of the virus, it would appear that this virus was not extremely virulent.

In experiment No. 4, 1 of the 6 control dogs showed symptoms of rabies on the sixteenth day, 4 on the seventeenth day, and 1 on the twentieth day. This incubation period would indicate a higher degree of virulence for this virus than for B. A. I. 474, yet 7 out of 10 dogs were protected against this virus, while 5 out of 6 dogs exposed to B. A. I. 474 virus died of rabies. These facts point to the possibility of the existence in this country of more than one strain of street virus.

In this event the extent to which the single-injection vaccine will assist in the control and eradication of rabies in dogs will depend in a large measure on the extent and distribution of the strain of virus designated B. A. I. 474 or similar strains, until such time as polyvalent vaccines can be prepared from more than the one strain of fixed virus as is now the case.

There is a possibility that the injection of a vaccine prepared according to the method of Umeno and Doi may result in death from fixed-virus infection, as seen in the case of dog 440.

The subcutaneous injection of fixed virus, even without attenuation, is generally considered to be harmless. This is undoubtedly true in general, but occasionally an individual may be encountered that is extremely susceptible and infection may result, even with an attenuated fixed virus. The percentage of such individuals, however, is believed to be small.

Umeno and Doi state that of the 31,307 vaccinated dogs there was one case of vaccination loss. It is presumed from this statement that the loss was due to fixed-virus infection following vaccination.

SUMMARY

The foregoing work may be summarized as follows:

The virus to which vaccinated dogs were exposed was the factor which determined the efficacy of the single-injection method of vaccination.

Against one virus no protection was afforded vaccinated animals.

Against two other viruses distinct protection was afforded.

The vaccines prepared in this country by commercial houses are all made from a strain of fixed virus having its origin in the Pasteur Institute of Paris, France.

There appears to be more than one strain of rabies street virus in this country.

THE STRAWBERRY ROOT LOUSE IN TENNESSEE [1]

By S. MARCOVITCH

Entomologist, Agricultural Experiment Station of the University of Tennessee

INTRODUCTION

In Tennessee, strawberries are classed as nursery stock and before they can be shipped an inspection is required for injurious insects, particularly for the strawberry root louse (*Aphis forbesi* Weed). Since no extended study of this insect in Tennessee had ever been made and since little is known concerning it in other States, except in Delaware, it seemed desirable to make a study of its habits and to determine if possible its economic importance. When one considers the vast quantities of strawberries grown in Tennessee, which is the largest strawberry-producing State in the Union, and the time consumed each year inspecting them, the need of a more definite knowledge of the economic importance of this insect becomes apparent.

GEOGRAPHIC RANGE

The strawberry root louse is a native insect confined to eastern North America, and has been reported injurious in Illinois, Ohio, Maryland, Tennessee, and Delaware, although it has recently been learned through correspondence that it is no longer so considered in the last-named State. In Tennessee it is present wherever strawberries are grown.

HOST PLANTS

The cultivated strawberry, *Fragaria* sp. appears to be the only host of *Aphis forbesi* Weed. Repeated efforts have been made to find it on other plants, but without success. Often the roots of various weeds are infested with lice very similar in appearance to the strawberry root louse, but close examination reveals them to be other species, usually the corn root louse, *Aphis maidiradicis*.

ECONOMIC IMPORTANCE

Plant lice in general are of great economic importance, yet there are many species that do little or no harm.

To obtain exact data in regard to injury caused by *Aphis forbesi* Weed, experiments were conducted in the field during 1921 and 1922. The vigor of a strawberry plant is indicated by the number of plants and runners which it produces. Plants artificially infested with lice were set out in March and a record was kept of the number of plants and runners produced. The plants used in the control plat were freed from any lice that may have been present by dipping them in a tobacco solution. The control plat was also frequently sprayed with tobacco to guard against infestation. The results obtained for the year 1922 are as follows: Of 12 infested mother plants the maximum number of plants produced by any one was 150, the minimum 12, and the average 47.6. Of 22 control mother plants the individual maximum was 120, the minimum 4, and the average 49.7 plants. The mother plants were set March 28 and the count of plants produced was made September 16, 1922. The difference in the percentage of plants produced by the infested mother plants and the controls was small, and could easily come within the limits of experimental error.

Practically the same results were obtained in 1921. Ladybird beetles often destroyed the lice on the crown of the plant, where they are frequently found. In such cases the plants were artificially reinfested.

To ascertain the general importance of the strawberry root louse a circular letter was sent to all the important strawberry-producing States. Replies from Iowa, Illinois, Ohio, New Jersey, Delaware, North Carolina, Georgia, Florida, Louisiana, Mississippi, and Arkansas indicated that the strawberry root louse was not considered injurious to the strawberry, or that little consideration was given to it. The only State reporting serious injury from this pest was Maryland, where it is considered "highly undesirable" in the light sandy soils, although no data were given. The strawberry soils of Ten-

[1] Received for publication June 11, 1924, issued, May, 1925.

nessee are mostly loams or silt loams, which may be one reason why the insect is less injurious in this State.

LIFE HISTORY

Unlike some other species of plant lice, the strawberry root louse spends its entire life cycle upon the strawberry, migrating only to other strawberry fields.

THE EGG

The insect passes the winter in the egg stage upon the pedicels of the leaves (pl. 1_e), although eggs are sometimes laid on the underside of the leaves. In 1921, at Knoxville, Tenn., hatching began on February 15, and in 1922 on February 22; hatching continues for about a month.

FIRST INSTAR (PL. 2, G)

Color characters: Upon hatching the new-born nymphs are pale green in color, with legs, antennae, and cornicles whitish. After a few hours the general coloration becomes greenish; head lighter, with two chitinized areas on either side of the median line; eyes reddish; antennae pale yellow, darker apically; tip of beak dusky; legs pale yellow; tarsi dusky; cornicles pale, darker at the tip.

Morphological characters: Body rectangular, flattened; antennae and legs robust as compared with the rest of the body; antennae four-jointed, with a sensorium present at the distal end of the third joint and at the proximal end of the flagellum of the fourth joint; cornicles short, about as broad as long.

Measurements: Length, 0.52 to 0.60 mm.; width, 0.28 to 0.30 mm.; antennae, 0.26 mm. (pl. 3, J); segment No. 1, 0.03 mm.; segment No. 2, 0.03 mm.; segment No. 3, 0.08 mm.; segment No. 4, 0.12 mm.; cornicles (pl. 3, K), length, 0.03 mm.; width, 0.03 mm.

SECOND INSTAR

This stage does not differ in general coloration from that of the first stage. The body becomes ovate in form and the antennae five-segmented. The cornicles also become more elongate, being about twice as long as wide.

Measurements: Length, 0.82 to 0.92 mm.; width, 0.40 to 0.44 mm.; antennae, 0.31 mm; segment No. 1, 0.03 mm.; segment No. 2, 0.03 mm.; segment No. 3, 0.06 mm.; segment No. 4, 0.04 mm.; segment No. 5, 0.15 mm.; cornicles, length, 0.05 mm.

STEM-MOTHER

After passing through five instars the stem-mother becomes mature in two to three weeks, depending upon the temperature. In 1921 stem-mothers were full grown as early as March 8. Upon maturity, often only a few hours after the last molt, they give birth to living young. In a study of the reproductive capacity of the stem-mothers, it was found that they were capable of giving birth to an average of two young per day for a period of 20 to 28 days. The highest number of young produced in one day was six.

The intitial feeding of the young stem-mothers takes place where they hatch, but after a short time they find their way to the tenderest leaves just coming out of the crown. Here they insert their beaks and suck the sap.

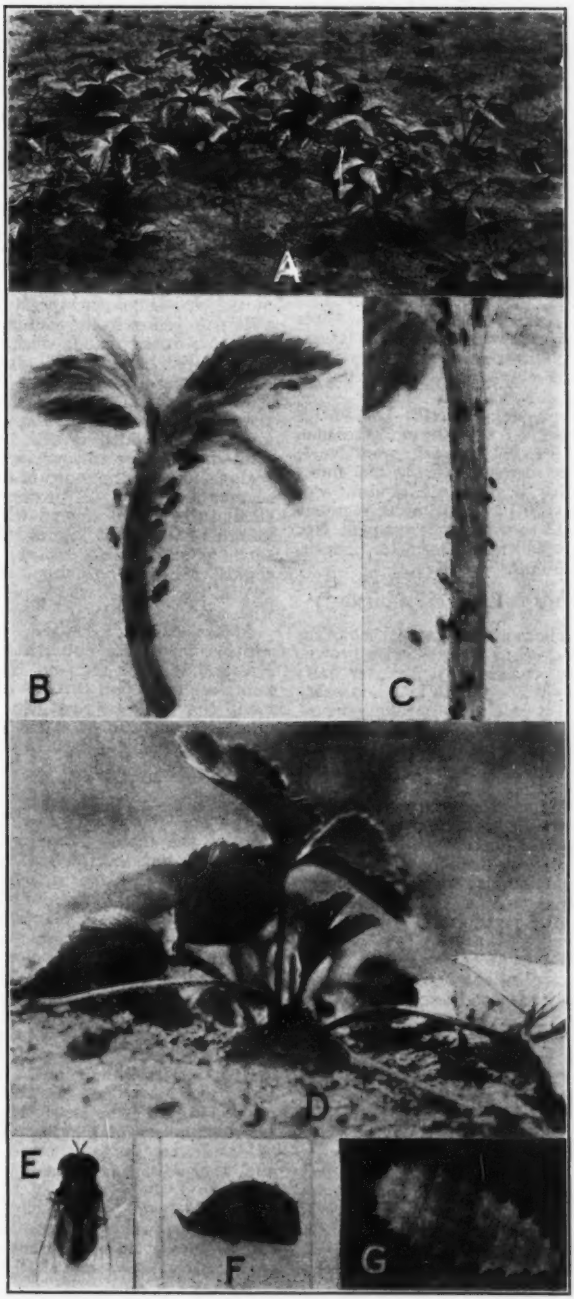
Color characters: The general coloring of the stem-mother is bluish-green, head greenish, with anterior portion lighter. Eyes dark red; antennae with segments 1, 2, and basal portion of 3, pale yellow; remainder of segments dark brown; beak yellowish, tip black, reaching to base of third pair of legs; legs yellowish; tarsi dusky; cornicles yellowish-brown, darker at tip; cauda yellowish brown, clothed with whitish hairs; posterior border of terminal abdominal segments, with a whitish pulverulent stripe; genital plate greenish-yellow; anal plate dark green.

Morphological characters: Body ovate, tending to pear-shape; antennae six-jointed, with segments 3 and 4 not differentiated; thorax with a prothoracic tubercle on each side; cornicles tubular, tapering distally.

Measurements: Length, 1.3 to 1.5 mm.; width, 0.74 to 0.78 mm.; antennae, segment No. 1, 0.05 mm.; segment No. 2, 0.04 mm.; segment No. 3, 0.22 to 0.24 mm.; segment No. 4, 0; segment No. 5, 0.10 to 0.11 mm.; segment No. 6, 0.06 to 0.18 to 0.20 mm.; cornicles, length, 0.19 to 0.21 mm.

EXPLANATORY LEGEND FOR PLATE 1

A.—Mother plant with 18 runners. Photograph made in midsummer from plot artificially infested with *Aphis forbesi*, showing vigorous condition
B.—Oviparous females of *Aphis forbesi* clustered on young tender leaf
C.—Egg of *Aphis forbesi* on pedicel of strawberry leaf
D.—Mound built by *Pheidole vinelandica* around pedicel of strawberry leaf protecting *Aphis forbesi*
E.—*Paragus tibialis:* Female, parasite on *Aphis forbesi*
F.—*Paragus tibialis:* Pupa
G.—*Paragus tibialis:* Larva



(For explanatory legend see p. 442)

WINGED VIVIPAROUS FEMALE (PL. 2, A)

Color characters: The general coloration is black. Head dark brown; eyes dark red, ocelli yellowish; antennae varying from pale yellow to dusky; beak yellowish, darker toward the tip; prothorax black, with anterior and posterior margins greenish, mesothorax and metathorax shining black, suffused with brown on the sides, legs yellowish; tarsi darker; abdomen greenish, with lateral margins of segments 1 to 5 with spotted black; cornicles brownish; cauda and genital plate green; anal plate black.

Morphological characters: Antennae six jointed, joints 3 and 4 sometimes indistinct, third segment with three sensoria; prothorax with two lateral tubercles; abdomen with a very small lateral tubercle; cornicles long, tubular, and flanged at the tip.

Measurements: Length, 1.3 to 1.8 mm., width, 0.7 to 0.8 mm., antennae, length, 0.9 to 1.1 mm.; segment No.1, 0.05 mm.; segment No. 2, 0.05 mm.; segment No. 3, 0.19 to 0.24 mm.; segment No. 4, 0.12 to 0.18 mm.; segment No. 5, 0.13 to 0.16 mm.; segment No. 6, 0.35 to 0.40 mm.; cornicles, length, 0.2 to 0.22 mm.

OVIPAROUS FEMALE (PL. 2, C AND E)

Color characters: General coloring yellowish-green; head yellowish-brown, sometimes greenish on posterior half; antennae with segments 1, 2, and basal portion of 3 pale yellow, remainder of segments darker; eyes dark red; beak greenish at base, pale yellow at middle, and dark at tip; legs pale yellow, tarsi darker; cornicles brownish, darker at tip; cauda dusky, clothed with whitish hairs; margins of abdomen greenish, with center of dorsum yellowish when the abdomen is distended with eggs; posterior abdominal segments more or less covered with a whitish pulverulence; genital plate greenish, with two circular yellowish areas on either side; anal plate dark green.

Morphological characters: The body is more elongate and narrower than in apterous viviparous female, tapering posteriorly. Antennae (pl. 3, H) six-jointed as in viviparous female; thorax with lateral tuberlces on prothorax; hind tibia (pl. 3, I) with about eight sensoria, not at all swollen, and hardly to be distinguished from the other forms; abdomen with a tubercle on either side; cornicles short, tubular, and flanged at the tip (pl. 3, E); genital plate more rounded than in the apterous viviparous female.

Measurements: Length, 1.2 to 1.5 mm.; width, 0.6 to 0.7 mm.; antennae, segment No. 1, 0.05 mm.; segment No. 2, 0.05 mm.; segment No. 3, 0.28 mm.; segment No. 4, 0; segment No. 5, 0.12 mm.; segment No. 6, 0.10 to 0.24 mm.; cornicles: length, 0.12 mm.

MALE (PL. 2, D AND F)

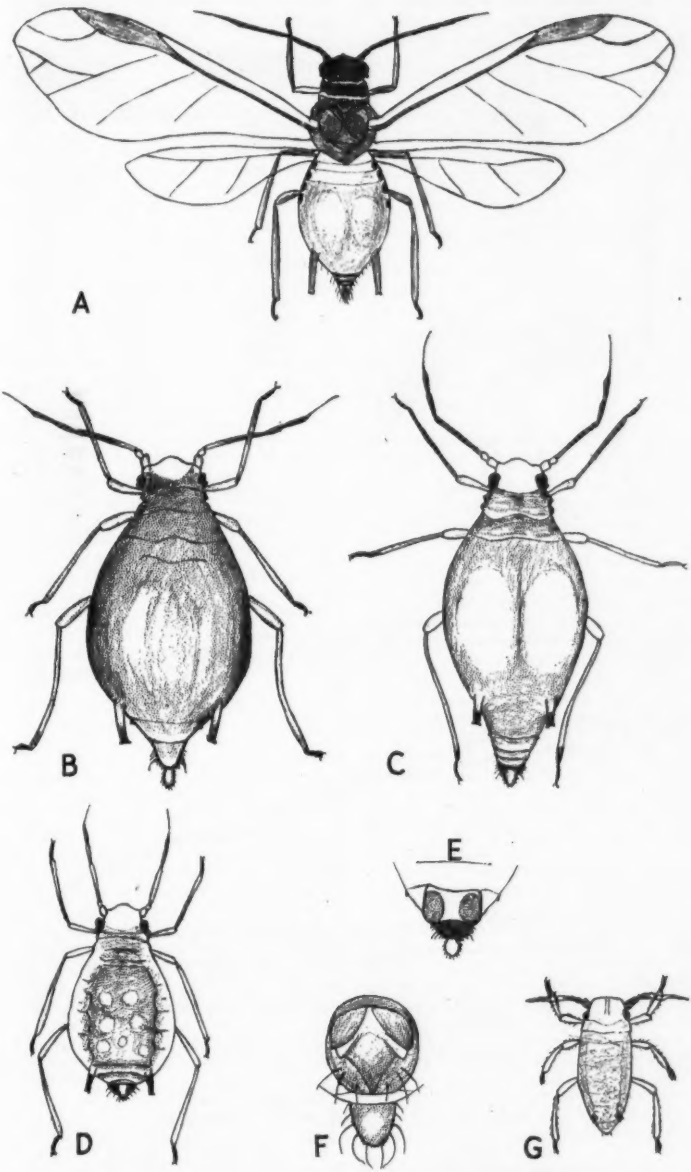
Color characters: General coloration brownish-yellow, mixed with green; antennae brownish-yellow, sensoria darker; head brownish, darker on posterior half; eyes dark brown, beak pale yellow, tip brown, thorax greenish; legs yellowish, with coxae, trochanters, and tarsi darker; cornicles brownish, darker at the tip; abdomen brownish spotted with pale yellow in the center, posterior segments dusky; cauda dusky, clothed with whitish hairs; genital plate brownish; anal plate black.

Morphological characters: Body short and broad; antennae about as long as the width of the body, six-jointed, with five to seven sensoria on segment 3, and one sensoria on each of segments 5 and 6; prothorax with small tubercles; abdomen with lateral margins flattened, a lateral tubercle on each side; cornicles short, tubular, slightly curved, and flanged at the tip; genital plate bears two claspers clothed with spines and hairs.

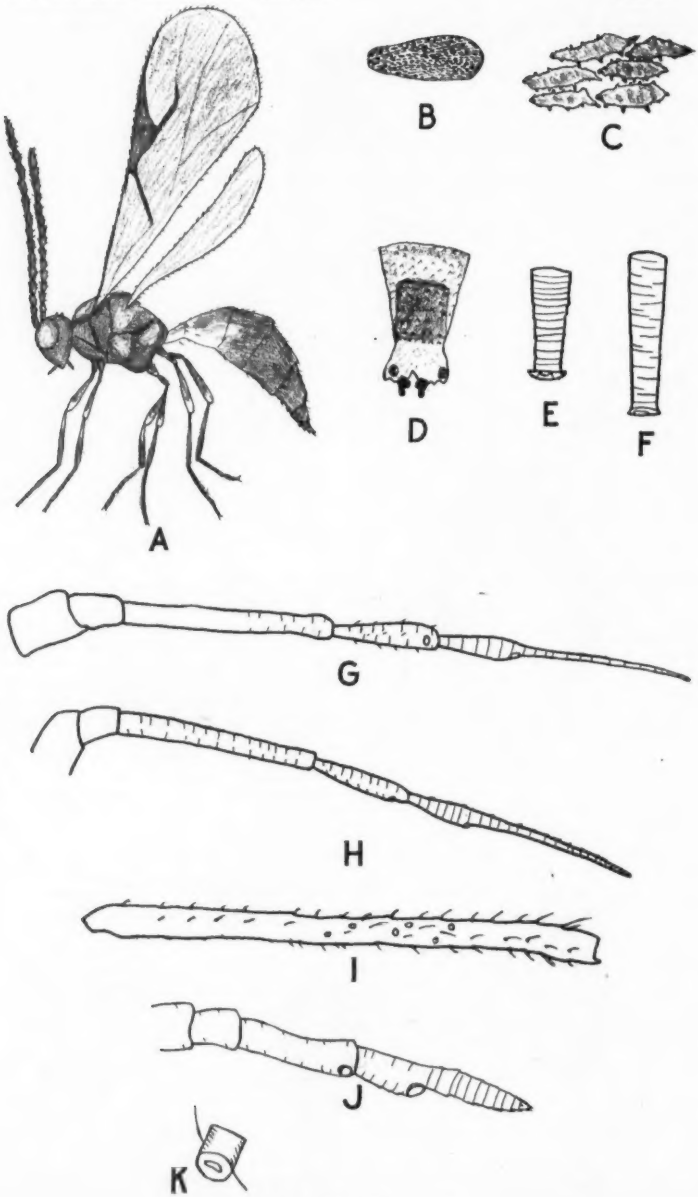
Measurements: Length, 1 mm.; width, 0.64 mm.; antennae, segment No. 1, 0.04 mm.; segment No. 2, 0.04 mm.; segment No. 3, 0.20 mm.; segment No. 4, 0; segment No. 5, 0.088 mm.; segment No. 6, 0.058+ to 0.18 mm.; cornicles, length, 0.12 mm.

SPRING FORMS

The majority of the second generation of the insect becomes wingless viviparous females (pl. 2, B, and pl. 3, F, G), although some winged ones are often present and distribute the species to other strawberry plants or beds. The young of the second generation are pale yellowish-green in color, in contrast to the dark green of the stem-mothers. The second and succeeding generations are usually found feeding on the pedicels of the young tender leaves, but are often deeply imbedded in the crown of the plant. They are sometimes located on the roots of the plants, but in Tennessee this is rare. Large numbers of plants were pulled to determine the location of the lice, and, except in a few cases, they were present on the pedicels of the young leaves. A generation of lice is produced about every 14 days throughout the summer, with winged ones appearing in each generation after the first.



A.—Winged viviparous female
B.—Wingless viviparous female
C.—Oviparous female
D —Male
E.—Posterior end of venter of oviparous female
F.—Posterior end of venter of male
G.—First instar of stem-mother



(For explanatory legend see p. 447)

In midsummer it is difficult to find any lice at all, owing to the activities of their natural enemies. Both young and mature forms were present on the rootlets as early as April 11.

THE TRUE SEXES

The true males and females make their appearance in Tennessee late in October, and may be found on the plants until February, when the eggs are ready to hatch. After maturing, the females deposit eggs, which carry the species over the winter. Although viviparous females may be present on the plants as late as December, they invariably die and do not assist in the reproduction of the species.

The males are small, wingless, and comparatively few in number. On one leaf pedicel there were counted 2 males and 22 oviparous females. The oviparous females can be readily recognized by their elongate appearance (pl. 1, B) and lighter color, caused by yellowish eggs showing through the chitin. In 1922 the first eggs were laid on November 9, although active egg laying does not begin until December. The eggs when first deposited are orange colored, and it is not until several days later that the characteristic shiny black color is assumed. The number of eggs deposited by a single female varies from four to eight.

FACTORS WHICH INFLUENCE THE APPEARANCE OF THE SEXES

With remarkably few exceptions, the true sexes in plant lice, including *Aphis forbesi*, make their appearance in the North Temperate Zone in the fall of the year and pass the winter in the egg stage. In the more southern localities plant lice generally reproduce throughout the year without the appearance of true sexes or eggs. Therefore, it is natural to conclude that the factor stimulating the formation of true sexes must be temperature; and such was assumed to be the case until it was noticed that the sexes made their appearance somewhat earlier on a plant growing in the laboratory than they did in the field. The laboratory air is warmer than that outside in the latter part of October and November, so that as far as the factor of temperature is concerned there seemed to be no correlation. There is, however, a marked difference between the intensity and duration of daily light exposure in the laboratory and the field. Garner and Allard [1] have shown that the length of daily light exposure influences the flowering and fruiting of plants, and since plant lice and plants both have asexual and sexual stages, which make their appearance at stated intervals, it occurred to the writer that possibly light rather than temperature was the determining factor in the formation of the sexes, and such was found to be the case.

PRODUCTION OF TRUE SEXES AND ITS RELATION TO THE SHORT DAYS OF FALL

One of the familiar phenomena of the Temperate Zone in the autumn is the shortening of the length of day. To create an artificial short day the potted plants used in the experiments were placed out of doors in a dark, ventilated chamber at 5 p. m. and kept there until 9.30 the next morning, when they were removed and placed in the light. The plants and plant lice were thus subjected each day to seven and one-half hours of light exposure. Any response on the part of the insects could hardly be attributed to temperature, since the temperature inside was but 2° to 3° F. higher than that outside. Single potted plants were also darkened by having inverted over them a larger pot or box.

In 1922 the plant with lice was subjected to a short day, beginning May 23. The first males and oviparous females were observed to make their appearance on September 18. Eggs were deposited on September 22, this being about seven weeks earlier than the first eggs are deposited in the field.

[1] GARNER, W. W., and ALLARD, H. S. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

EXPLANATORY LEGEND FOR PLATE 3

A.—*Diaeretus fusicornis*
B.—*Paragus tibialis:* Egg
C.—*Paragus tibialis:* Egg under high magnification showing sculpturing
D.—*Paragus tibialis:* Caudal appendage
E.—*Aphis forbesi:* Cornicle of oviparous female
F.—*Aphis forbesi:* Cornicle of wingless viviparous female
G.—*Aphis forbesi:* Antenna of wingless viviparous female
H.—*Aphis forbesi:* Antenna of oviparous female.
I.—*Aphis forbesi:* Hind tibia of oviparous female
J.—*Aphis forbesi:* Antenna of first instar stem-mother
K.—*Aphis forbesi:* Cornicle of first instar stem-mother

In 1923 the plants were given a short day, beginning February 23, at which time the eggs were already hatching. By May 7 males and oviparous females had made their appearance, and the first eggs were deposited on May 22. In another experiment, beginning March 21, a plant with lice in the second generation was subjected to a short day, and by May 12 the oviparous females had made their appearance.

THE TRUE SEXES AS INFLUENCED BY A LONG DAY

According to the above experiments the production of the true sexes appears to be governed by the short days of the fall. How, then, would a daily light exposure of 15 hours or more act, and would the production of viviparous forms continue? To determine this point, some strawberry plants infested with lice were placed out of doors September 4 and given 15 hours of daily light exposure by means of a 60-watt electric light, which hung about 2 feet from the plants. At this distance the light did not raise the temperature of the air around the plants, as shown by several tests. A control plant was placed inside the laboratory, next to the window at the same time. On October 6 viviparous females made their appearance on the control plant, but were not yet present on the plants given the long day. On November 5 eggs were found on the control.

When examined on December 22, viviparous forms only were still present out of doors on the plants receiving the long exposure. However, the sexes did make their first appearance on January 4, 1924. This would indicate that a long day may successfully inhibit the formation of the true sexes, provided a certain temperature level is maintained. The first 15 days of December averaged 40° F. and it is very likely that it was in this period that sex formation was initiated. In addition to a certain temperature level, possibly minimum temperatures may be factors. A minimum temperature of 28° on November 9 and 10, and 20° on December 15 was recorded.

Garner and Allard [2] have found that biennials will behave as annuals when subjected to a combination of long days and a lower temperature level. Lower temperatures alone would not accomplish this result.

It should be noted that the strawberry plants bearing aphids were given a long daily light exposure beginning September 4. The experiment was started early enough to insure if possible against the production of the sexes. In 1922, strawberry plants were given a long daily exposure beginning October 12, and a few sexed individuals appeared two weeks later. This shows that once the tendency toward true sex production is initiated, it can not be done away with. However, in the early part of September that tendency is still absent in *Aphis forbesi* in Tennessee.

RELATION OF ANTS TO THE APHIS

Sanderson [3] records that the ant, *Lasius alienus*, attends the strawberry root louse in Delaware and carries it down to the roots of the plants, especially where the soil is sandy. In Tennessee various species of ants have been observed to care for the lice, but the species that is by far mostly commonly found is the little brown ant, *Pheidole vinelandica*, as determined by W. M. Wheeler. This species may be found about strawberry plants in March and up to January. Its method of tending the lice in order to obtain honeydew is interesting. Since the lice are most often clustered on the pedicels of the small, tender leaves, the ants protect them by building small craterlike mounds of dirt about the crown, so that the pedicels of the young leaves may be entirely covered and concealed from view, as shown in Plate 1, D. These little craters are often 2½ inches high, and sometimes completely cover the crown of the plant. In such cases lice may be found feeding on the crown of the strawberry.

NATURAL ENEMIES

The strawberry root louse will sometimes increase in numbers to such an extent that it becomes noticeable, but in most cases its natural enemies keep it in check. Several different species of these were bred, among them a syrphid that had never been reported as preying upon the strawberry root louse, and an undetermined chalcid. Parasites were bred as late as December 14 and as early as May 14. Ladybird beetles, such as *Hippodamia convergens* and *Coccinella novemnotato*, were frequently found upon the root louse, as well as a larva

[2] TAYLOR, W. A. REPORT OF THE CHIEF OF THE BUREAU OF PLANT INDUSTRY. U. S. Dept. Agr. Ann. Rpt. 1922/23: 255–288. 1924.

[3] SANDERSON, E. D. THE STRAWBERRY ROOT LOUSE. Del. Agr. Exp. Sta. Bul. 49: 1–13, illus. 1900.

of one of the Chrysopidae. Sanderson[4] records several hymenopterous parasites, among which are *Lysiphlebus myzi* Ashm., *L. salicaphidis* Ashm., *Lygocerus stigmatatus* Sag., and *Adialatus densleonis* Ashm. A louse attacked by parasites appears plump and greenish in color. As the parasite grows the body of the louse becomes much distended, and upon maturity nothing is left of the host except the straw-colored chitinous envelope.

DIARETUS FUSCICORNIS ASHM.

This little braconid (pl. 3, A) as determined by A. B. Gahan, was frequently bred from lice clustered on the pedicels of the leaves, and undoubtedly helps to keep the lice in check. In color, it is shining black, legs yellowish, mixed with brown. Antennae of male 15-jointed, of female 13-jointed. Length 1.6 mm.

PARAGUS TIBIALIS

This syrphid (pl. 1, E, F, G and pl. 3, B, C, D) appears to be the most constant and efficient enemy of the strawberry root louse in Tennessee. The larvae were found in the field as early as April 18. A larva was observed to devour seven lice in five minutes in the laboratory. Larvae collected on May 20 pupated May 22, and the adults emerged May 29. The puparium is inconspicuous on the under side of the leaves, and rather resembles a piece of dirt.

EGG.—Chalk-white in color. In outline, subcylindrical and ovate; truncated at the narrow end and rounded at the other. The surface of the egg is covered with very fine microscopic elevations arranged in lines, which under high magnification are seen to possess projecting arms, as shown in pl. 3, C. Length, 1 mm.; width, 0.40 mm.

LARVA.—General coloring pale yellow, mixed with patches of brown; posterior respiratory appendages dark brown at base, yellowish on apical third. Dorsal spiracular spine brownish. Surface of skin covered with minute, wartlike elevations. The spines on segment 4 appear equal. Beyond this segment the dorso-lateral spines are the longest, the dorsal spines small. Dorsal spiracular spine large, concave lateral, bifurcate. Length, 6 to 7 mm. Length of posterior respiratory appendage, 0.46 mm.

ADULT.—General coloring black, frequently reddish on the abdomen. Both male and female with a median black band on the face. Length, 3 to 5 mm.

SUMMARY

Since strawberries are classed as nursery stock in some States and required to be inspected for the strawberry root louse, *Aphis forbesi* Weed, it becomes necessary to determine more exactly its economic importance. Observation and experiments since 1919 show that *Aphis forbesi* is present in all the strawberry-growing areas of Tennessee and that it can not be classed as highly injurious in that State. It is not a leaf curler and apparently injects no toxin into the host plant.

Plants were artificially infested with the root louse and kept so throughout the summer to determine the effects on the production of runners. These plants each produced an average of 47.6 plants as compared with 49.7 plants produced by the controls.

Aphis forbesi passes the winter in the egg stage on the pedicels of the leaves. Hatching begins about February 15 and continues for about a month. Reproduction continues viviparously throughout the summer and as late as November. In Tennessee the lice remain mostly on the pedicels of the leaves and around the crown of the plant. Only rarely are they found on the roots. The ant, *Pheidole vinelandica*, is most commonly associated with *Aphis forbesi*.

The true sexes first make their appearance in October and may be found on the plants until February.

In a study of the factors influencing the production of the true sexes in *A. forbesi*, it was found that the relative length of exposure to daily light appears to be an important factor. By giving a daily short exposure of seven and one-half hours, beginning February 23, the sexes made their appearance on May 7. Eggs were deposited on May 22.

During the fall months of 1923 when the plants were given a long daily light exposure of 15 hours out of doors, the aphids continued to reproduce viviparously as late as January 4, 1924, when the first true sexes made their appearance. On the control plant the sexes appeared October 6. It is possible that a combination of long days and a temperature of 50° F. or higher might have sufficed to keep the sexes from appearing.

The strawberry root louse has many natural enemies, among the most important of which is the little syrphid known as *Paragus tibialis*. Other natural enemies include several species of Braconidae, the most common being *Diaeretus fuscicornis*. Several Coccinelidae and Chrysopidae may frequently be found preying on the lice.

[4] SANDERSON, E. D. REPORT OF THE ENTOMOLOGIST. The strawberry root louse. Del. Agr. Exp. Sta. Ann. Rpt. (1899/1900) 12: 143-169, illus. 1901.

THE EFFECT OF SULPHUR AND GYPSUM ON THE FERTILITY ELEMENTS OF PALOUSE SILT LOAM[1]

By LEWIS W. ERDMAN[2]
Washington Agricultural Experiment Station

INTRODUCTION

During the past few years some soils have been found to be quite low in total sulphur, and the use of sulphur as a fertilizer has been suggested as a means of remedying this condition. Most of the experiments with sulphur have had certain objects in view: (1) To study its effect on the growth and composition of crops; (2) to determine its effectiveness when inoculated with sulphur-oxidizing bacteria and composted with manure and rock phosphate in rendering available the phosphorus in the rock phosphate; and (3) to determine its effectiveness when composted with manure and greensand, in making the potassium in the greensand available for crops. Very little attention has been given to the direct effect of sulphur on the fertility elements in the soil. From the soil fertility point of view, this problem is of unusual significance, because the future value of sulphur as a fertilizer will depend not only upon its ability to increase crop growth, but also upon the reactions which it brings about in the soil, and especially upon its effects on those elements which have a direct bearing on fertility.

The present investigation was planned to ascertain to what extent the sulphur, potassium, phosphorus, nitrogen, calcium, and magnesium of the Palouse silt loam, an important soil type in Washington, are affected by applications of uninoculated sulphur, inoculated sulphur, and gypsum.

EXPERIMENTAL WORK

The soil used was a typical Palouse silt loam secured from an alfalfa pasture on the experimental farm of the State College of Washington. Wheat is grown extensively on this particular soil. It is derived mainly from basaltic rock and, judging from the following chemical analysis, may be considered of good fertility.

Pounds per 2,000,000 pounds of soil: Nitrogen, 2,900; phosphorus, 1,280; potassium, 42,400; sulphur, 960; calcium, 29,200; magnesium, 20,200.

Twenty-four ordinary clay flowerpots (inside diameter at top 12 inches, at bottom 8.75 inches, and depth 8.5 inches) were used for this work. These were divided into two series of 12 each, the first series being used for experiment 1, and the other for experiment 2. Each pot was thoroughly paraffined on the inside, and in the bottom was placed a paraffined cork stopper through which a short glass tube was inserted to permit the removal of drainage water. The outlet of the tube was covered with a very small inverted clay pot to prevent the soil from clogging the opening.

EXPERIMENT 1

In an open field on the college farm a stand was built large enough to hold 12 pots and just high enough to allow 2.25-liter acid bottles to be placed beneath. The glass tube leading from each pot entered one of these bottles, the mouth of which was covered to exclude rain and dust particles. On November 27, 1922, 18½ lbs. of soil (moisture-free basis) was placed in each pot. This amount of soil approximated the ordinary depth of surface soil (6⅝ inches). The following treatments, made in duplicate, were thoroughly mixed in the upper 2 inches of soil on December 5, 1922.

[1] Received for publication June 17, 1924; issued May, 1925. Published with the approval of the director of the Washington Agricultural Experiment Station as Scientific Paper No. 109. The work reported in this paper was done in the laboratories of the Division of Chemistry of the Washington station under the direction of Dr. J. R. Neller.

[2] Formerly research fellow, National Research Council Sulphur Fellowship. Now assistant professor of soils and assistant chief in soil bacteriology, Iowa State College. The writer wishes to acknowledge his indebtedness to the Sulphur Fellowship Committee of the National Research Council for the appointment to this fellowship; to Dr. J. R. Neller for many helpful suggestions; and to Dr. P. E. Brown for reading the manuscript.

Pot No.	Treatment	Pounds per acre
1 and 2	Control	
3 and 4	Uninoculated sulphur	186
5 and 6	Inoculated sulphur	189
7 and 8	Uninoculated sulphur	1,000
9 and 10	Inoculated sulphur	1,015
11 and 12	Gypsum	1,000

The soils in pots Nos. 3, 4, 5, 6, 11, and 12 received equivalent amounts of sulphur, the calculations being made on the basis that chemically pure gypsum, which was used in these experiments, contained 18.6 per cent of sulphur. The samples of uninoculated and inoculated sulphur were obtained from the Texas Gulf Sulphur Co. Inoculated sulphur is supposed to contain large numbers of efficient sulphur-oxidizing bacteria which give it the ability to act quicker than uninoculated sulphur.

The soils in the pots placed in the field were exposed to natural weather conditions and remained undisturbed from December 5, 1922, to June 18, 1923. On the latter date a composite soil sample was taken from each pot, and water extractions were made for the purpose of studying the amount of water-soluble plant food material in the soils at the close of the experiment. During the progress of the experiment the drainage water from each pot for each month was collected, filtered through Buchner funnels, and stored in large bottles in the laboratory. A small quantity of toluol was added to each bottle to inhibit bacterial action.

EXPERIMENT 2

On December 9, 1922, an exact duplication of experiment 1 was started in the greenhouse to verify and elaborate the results obtained from the soils exposed to natural weather conditions. After the treatments were made the soils were adjusted to the optimum moisture content, and for a period of 72 days the moisture content was maintained by additions of distilled water to weight. At the end of this time all of the soils were flooded with definite amounts of distilled water for 4 consecutive days, and the leachings thus obtained were treated and analyzed as in the case of the drainage water secured from the pots in experiment 1. Soil extracts wese made from these soils after they were allowed to dry.

ANALYTICAL WORK

The analytical work for these experiments involved (1) the analyses of the drainage water obtained form the pots which were exposed to field conditions; (2) the analyses of the leachings from the pots which were placed in the greenhouse; and (3) the analyses of the water extracts made from the soils at the close of both experiments. These analyses included determinations of sulphur, nitrogen, phosphorous, potassium, calcium, and magnesium. Colorimetric hydrogen-ion determinations were made on samples of soil taken from the pots at the conclusion of both experiments and also on samples of the drainage water obtained from each pot for the month of June, 1923.

A rain gauge of the standard United States Weather Bureau type was placed beside the pots used in experiment 1. Records were kept of the amount of precipitation, and samples of rain water for each month were analyzed for total sulphur. A few ammonia and nitrate determinations were made on the first samples, but since only traces of these compounds were present the study of nitrogen in the rain water was abandoned.

DISCUSSION OF RESULTS

This study was limited to the surface 6⅔ inches of soil, and it should be understood that the entire amount of elements reported in the drainage water was not necessarily totally lost to crops. Under actual field conditions some of the water-soluble elements may be returned to the surface soil from the deeper soil layers by capillarity. It is believed, however, that the results secured are sufficient to aid materially in obtaining a better understanding of the probable effects of sulphur on soil fertility.

Climatic conditions at the Washington Agricultural Experiment Station are ideal for lysimeter studies. The annual average rainfall is 21.49 inches. The heavy rains, when leaching is most apt to occur, come during the period extending from November to June. The season during which these experiments were made was exceptionally dry in the fall, and the winter rains did not begin until early in December, or immediately after the pots were placed in the field. Therefore as far as the percolation studies are concerned this investigation may be considered as representing the work of one year.

The rainfall and the total sulphur of the rain and snow covering the period from November 29, 1922, to June 18, 1923, are found in Table I.

TABLE I.—*Amount of rainfall, and sulphur in rain and snow from November 29, 1922, to June 18, 1923*

Date	Amount of rainfall	Pounds per acre	
		Sulphur	Calculated as SO_3 sulphur
	Inches		
1922. No. and Dec.	4.18	0.83	1.87
1923. Jan.	3.75	1.11	2.77
Feb.	.95	.54	1.35
Mar.	.54	.48	1.20
Apr.	.88	.29	.72
May	1.30	.70	1.75
June	3.25		
Total	14.85	3.95	9.66

The total amount of rainfall for the eight months was 14.85 inches, which indicates that the rainy season for 1922 to 1923 was not quite up to the average. For this reason the results obtained from this study are probably all the more valuable. The total amount of sulphur added to an acre of soil in the rain and snow for this period (exclusive of the June sample which was contaminated by birds) was 3.95 lbs. If 2 lbs. of sulphur is allowed for the months of June and October, which seems to be a fair estimate, judging from the other analyses this would make a total of about 6 lbs. of sulphur per acre reaching the soil through the annual precipitation. This quantity is very much below that reported by some other investigators. The writer [3] found an average of about 15 lbs. of sulphur added to an acre of soil in Iowa by the rain water. Other analyses of the rain falling in cities and towns vary from 25 to over 300 lbs. of sulphur per acre annually. The results obtained for the Palouse country are probably low, because this section is far removed from the great coal-consuming centers which furnish the atmosphere with the greater portion of its sulphur.

Table II shows the amount of drainage water and leachings collected from the pots in both experiments.

TABLE II.—*Amount of drainage and leachings*

Pot No	Treatment (pounds per acre)	Experiment 1 (field pots)				Experiment 2 (greenhouse pots), total leachings
		December	January and February	March and June	Total for five months	
		C.c.	*C.c.*	*C.c.*	*C.c.*	*C.c.*
1	Control	1,930	5,835	2,655	10,420	6,555
2	do	1,869	4,515	2,640	9,024	6,945
	Average	1,900	5,175	2,648	9,722	6,750
3	Uninoculated sulphur 186	2,329	6,965	3,215	12,509	7,300
4	do	2,245	5,200	2,875	10,320	6,460
	Average	2,287	6,083	3,045	11,415	6,880
5	Inoculated sulphur 189	2,305	5,960	3,210	11,475	7,090
6	do	1,904	5,250	2,870	10,024	6,705
	Average	2,105	5,605	3,040	10,750	6,898
7	Uninoculated sulphur 1000	2,379	6,690	3,180	12,249	6,725
8	do	2,057	4,095	2,660	8,812	6,610
	Average	2,218	5,393	2,920	10,531	6,668
9	Inoculated sulphur 1015	2,269	5,380	2,960	10,609	6,430
10	do	2,107	4,937	2,765	9,809	6,065
	Average	2,188	5,159	2,863	10,209	6,248
11	Gypsum 1000	2,289	5,515	2,800	10,604	6,580
12	do	2,127	5,167	2,725	10,019	6,630
	Average	2,208	5,341	2,763	10,312	6,605

[3] ERDMAN, L. W. THE SULPHUR CONTENT OF RAINWATER. Soil Sci. 14: 363-367. 1922.

A striking uniformity is observed in the amount of drainage water leached through each pot. The small discrepancies may readily be accounted for by the fact that the bottoms of some of the clay pots were slightly raised in the center. This would allow a certain amount of water to accumulate and remain in these pots after each rain. The drainage water for January and February was combined for analysis, and likewise the March drainage was added to the June drainage water. Owing to the small rainfall for April and May there was no percolation of water through the pots for these months.

SULPHUR IN DRAINAGE WATER AND LEACHINGS

It is not known what proportion of the total sulphur in the soil is present in the organic form and how much is free or combined with the soil minerals. In soils well supplied with organic matter probably a large portion of the sulphur is organic, because sulphur forms part of the protein molecule and is therefore present in all plants and animals. The decomposition of organic sulphur compounds results in the formation of thiosulphates, hydrogen sulphide, and free sulphur, and all of these substances may be oxidized to sulphates by the sulphur-oxidizing microorganisms. This process, termed sulphofication, is probably responsible for the large losses of sulphur in the drainage water. Lyon and Bizzell [4] have tabulated the results of a number of investigators and found that the loss of sulphur in drainage water varied from 8 to 281 lbs. per acre, depending upon the type of soil and the kind of cropping system followed.

By applying elemental sulphur to soils and measuring the sulphates coming through in the drainage water, a definite idea of the rate and extent of sulphofication may be determined. In Table III are found the results of the sulphur analyses of the drainage water and leachings obtained from the variously treated soils in both experiments.

TABLE III.—*Amount of sulphur in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—sulphur in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—sulphur in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble sulphur	Leachings	Soil extracts	Total water-soluble sulphur
1	Control	11.7	14.5	21.1	47.3	29.0	76.3	33.2	10.9	44.1
2	do	8.5	4.7	22.2	35.4	22.0	57.4	41.3	12.0	53.3
	Average	10.1	9.6	21.6	41.3	25.9	66.8	37.2	11.4	48.7
3	Uninoculated sulphur 186	9.8	11.1	66.9	87.8	141.6	229.4	151.5	108.2	259.7
4	do	11.3	13.1	106.9	131.3	156.5	287.8	200.7	80.4	281.1
	Average	10.5	12.1	86.9	109.5	149.0	258.6	176.1	94.3	270.4
5	Inoculated sulphur 189	11.2	10.2	77.5	98.9	138.9	237.8	169.9	114.3	284.2
6	do	9.4	10.4	102.0	121.8	163.9	285.7	138.1	87.2	225.3
	Average	10.3	10.3	89.7	110.3	151.4	261.7	154.0	100.7	254.7
7	Uninoculated sulphur 1000	8.7	9.8	156.9	175.4	416.1	591.5	544.6	746.5	1,291.1
8	do	9.3	17.8	146.3	173.4	456.6	630.0	515.6	649.5	1,165.1
	Average	9.0	13.8	151.6	174.4	436.3	610.7	530.1	698.0	1,228.1
9	Inoculated sulphur 1015	13.2	13.4	182.5	209.1	483.5	692.6	552.4	711.1	1,263.5
10	do	10.1	14.0	154.5	178.6	472.0	650.6	529.8	598.4	1,128.2
	Average	11.6	13.7	168.5	193.8	477.7	671.6	541.1	654.7	1,195.8
11	Gypsum 1000	100.2	169.1	30.5	299.8	47.2	347.0	98.6	69.9	168.5
12	do	104.8	180.4	30.3	315.5	35.0	350.5	178.4	65.4	243.8
	Average	102.5	174.7	30.4	307.6	41.1	348.7	138.5	67.6	206.1

[4] LYON, T. L., and BIZZELL, J. A. LYSIMETER EXPERIMENTS. RECORDS FOR TANKS 1 TO 12 DURING THE YEARS 1910 TO 1914 INCLUSIVE. N. Y. Cornell Agr. Exp. Sta. Mem. 12, 115 p., illus. 1918.

These data reveal some interesting facts. Considering first, the results of experiment 1, it may be noticed that after three months there was only a slight oxidation of the elemental sulphur, owing possibly to the cold temperatures prevailing during this time. From February to June the temperature was more favorable for the activity of soil organisms, and a considerable portion of the elemental sulphur from both the small and large applications was changed to the sulphate form. Practically the same amount of sulphates was produced from the small applications of uninoculated and inoculated sulphur. Slightly more sulphates were formed from the large application of inoculated sulphur than from the same amount of uninoculated sulphur.

It was realized that the amount of rainfall percolating through the soil in the pots was not sufficient to remove all of the sulphur which had been changed to sulphates. Therefore, at the conclusion of the experiments, soil extracts were made from a composite soil sample from each pot in order to throw more light on the changes that the elemental sulphur had undergone in the soil. The data obtained from these extracts were added to the amount of sulphur recovered in the drainage in order to get the total water-soluble sulphur. By examining the latter data it is noted, first, that after seven months approximately 200 lbs. of sulphate sulphur was obtained from the low-sulphur pots Nos. 3, 4, 5, and 6, and, second, that a little more than one-half of the 1,000 pounds of sulphur added to pots Nos. 7, 8, 9 and 10 was recovered as sulphates.

These facts indicate that this soil has a very marked sulphofying power, which probably explains why the inoculated and uninoculated sulphur treatments produced almost the same quantity of sulphates.

It may be well to recall that the soil in the pots in the greenhouse was maintained at optimum moisture conditions for a period of 72 days. During this time conditions should have been extremely favorable for optimum microorganic activity, and the data given for experiment 2 show that such conditions did exist. The results for the small sulphur treatments agree almost perfectly with the data obtained from experiment 1. In the case of the larger sulphur additions it is surprising to note that the total 1,000 lbs. of sulphur which was originally added was recovered as sulphates after two and one-half months. The large sulphur treatments seemed to have had a marked influence also on sulphofication, as is shown by the increase in sulphur obtained over the amount added and the amount recovered from the untreated soils. These data also show that for this soil the uninoculated sulphur is just as effective as the inoculated sulphur in producing available sulphur for plants.

A striking feature of the December analysis is that practically one-half of the sulphur added in the 1,000 lbs. of gypsum leached through the surface soil at the end of one month. After three months all of the sulphur originally added, and an appreciable quantity besides, appeared in the drainage water. By June the gypsum-treated soil showed a still further increase in sulphur in the drainage water when compared with the control. These results would seem to indicate that gypsum greatly increased sulphofication in this soil. The results obtained in the greenhouse for the soil treated with gypsum, however, do not agree with these findings, but show on the other hand that gypsum did not increase sulphofication. The data obtained from the field pots treated with gypsum present a strong argument for the spring application of gypsum, because if applied in the fall it may be largely lost through leaching before the crop has a chance to make use of it.

EFFECT OF SULPHUR AND GYPSUM ON POTASSIUM

The results of the analyses of the drainage water and leachings for potassium are given in Table IV.

An examination of the amount of potassium leached from the control soil indicates that the Palouse silt loam is not deficient in available potassium. All of the sulphur treatments caused small but decided increases in the amount of potassium appearing in the drainage water. The results of both experiments confirm this statement, although it is not known why so much more water-soluble potassium was present in the drainage water and water extracts from the field soils than was the case in the soils in the greenhouse. The process of making potassium available is certainly correlated with sulphofication, because, as shown by the analyses of the drainage water obtained during the different months, no effect on soil potassium was produced until the end of June, or after appreciable quantities of sulphur had been oxidized. This statement is very

TABLE IV.—*Amount of potassium in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—potassium in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—potassium in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble potassium	Leachings	Soil extracts	Total water-soluble potassium
1	Control	24.1	35.9	120.8	180.8	244.4	425.2	27.5	156.3	183.8
2	do	25.0	22.9	202.7	250.6	231.5	482.1	56.1	157.2	213.3
	Average	24.5	29.4	161.7	215.7	237.9	453.6	41.8	156.7	198.5
3	Uninoculated sulphur 186	26.8	32.8	160.7	220.3	278.1	498.4	80.0	176.0	256.0
4	do	24.0	25.4	195.8	245.2	262.1	507.3	82.5	169.8	252.3
	Average	25.4	29.1	178.2	232.7	270.1	502.8	81.2	172.9	254.1
5	Inoculated sulphur 189	25.0	27.1	181.0	233.1	254.0	487.1	75.6	182.4	258.0
6	do	21.6	24.5	181.1	227.2	242.8	470.0	74.5	171.2	245.7
	Average	23.3	25.8	181.0	230.1	248.4	478.5	75.0	176.8	251.8
7	Uninoculated sulphur 1000	25.7	31.9	215.9	273.5	302.2	575.7	131.3	226.7	358.0
8	do	25.3	23.5	227.9	273.5	287.8	561.3	131.9	201.6	333.5
	Average	25.5	27.7	221.9	275.1	295.0	568.5	131.6	214.1	345.7
9	Inoculated sulphur 1015	28.7	24.5	268.1	321.3	285.4	606.7	131.4	234.4	365.8
10	do	26.3	31.4	235.3	293.0	298.2	591.2	113.4	200.2	313.6
	Average	27.5	27.9	251.7	307.1	291.8	598.9	122.4	217.3	339.7
11	Gypsum 1000	38.7	54.6	106.9	200.2	241.1	441.3	60.8	172.2	233.0
12	do	41.1	58.9	114.1	214.1	233.1	447.2	84.6	183.3	267.9
	Average	39.9	56.7	110.5	207.1	237.1	444.2	72.7	177.7	250.4

strikingly brought out by the data given for the high sulphur treatments. In this connection Ames [5] suggested that the liberation of potassium was brought about by salts formed rather than by the direct action of acidity, developed from nitrification and sulphofication, on insoluble potassium compounds. That this assumption holds true for these experiments will be shown later, when the hydrogen-ion concentrations of the soils are considered.

The inoculated and uninoculated sulphur treatments showed practically the same effect in liberating the insoluble soil potassium.

The data given for the analyses of the drainage water from the soils treated with gypsum indicate that for the first three months gypsum caused an increase in water-soluble potassium, but, on the other hand, the data for the total water-soluble potassium show that gypsum slightly decreased the amount of potassium going into solution. A correct interpretation of the effect of gypsum on soil potassium in experiment 1 is made impossible by the seemingly high analysis reported for the control soil No. 2 for the March and June drainage. The data given for experiment 2 show that under greenhouse conditions gypsum brought about a distinct increase in the amount of potassium found in the leachings and also in the soil extracts.

EFFECT OF SULPHUR AND GYPSUM ON CALCIUM

Tottingham and Hart [6] summed up their work on sulphur and sulphur composts with the statement that "adequate consideration of the use of sulphur as a fertilizer must recognize its tendency to deplete the stock of

[5] AMES, J. W. SOLVENT ACTION OF NITRIFICATION AND SULFOFICATION. Ohio Agr. Exp. Sta. Bul. 351, p. 221–257. 1921.

[6] TOTTINGHAM, W. E., and HART, E. B. SULFUR AND SULFUR COMPOSTS IN RELATION TO PLANT NUTRITION. Soil Sci. 11: 49–65, illus. 1921.

CaO, P_2O_5, and other soil constituents." The extent to which the supply of calcium in Palouse silt loam may be depleted by the use of sulphur is brought out in the data presented in Table V.

doubt on the accuracy of all of the calcium data obtained from the soil extracts in experiment 1.

The results obtained from experiment 2 show that all of the sulphur treatments caused a marked loss of

TABLE V.—*Amount of calcium in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—calcium in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pot)—calcium in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble calcium	Leachings	Soil extracts	Total water-soluble calcium
1	Control	52.9	43.9	36.1	132.9	625.7	758.6	56.4	556.4	612.8
2	do	41.8	32.4	62.7	136.9	487.3	624.2	78.6	574.9	653.5
	Average	47.3	38.1	49.4	134.9	556.5	691.4	67.5	565.6	633.1
3	Uninoculated sulphur 186	44.0	41.4	56.3	141.7	547.2	688.9	138.2	579.8	718.0
4	do	44.7	33.2	89.5	167.4	495.6	663.0	176.0	552.6	728.6
	Average	44.3	37.3	72.9	154.5	521.4	675.9	157.1	566.2	723.3
5	Inoculated sulphur 189	46.1	36.9	68.3	151.3	543.1	694.4	150.0	635.6	785.6
6	do	39.9	30.5	91.7	162.1	526.6	688.7	126.3	552.6	678.9
	Average	43.0	33.7	80.0	156.7	534.8	691.5	138.1	594.1	732.2
7	Uninoculated sulphur 1000	44.8	38.2	129.4	212.4	404.7	617.1	458.2	646.7	1,104.9
8	do	42.8	30.2	94.2	167.2	353.1	520.3	432.8	548.9	981.7
	Average	43.8	34.2	111.8	189.8	378.9	568.7	445.5	597.8	1,043.3
9	Inoculated sulphur 1015	61.1	36.2	163.7	261.0	351.1	612.1	455.8	557.5	1,013.3
10	do	46.3	35.0	103.9	185.2	417.1	602.3	421.0	495.6	916.6
	Average	53.7	35.6	133.8	223.1	384.1	607.2	438.4	526.5	964.9
11	Gypsum 1000	107.6	141.0	34.4	283.0	561.7	844.7	90.6	631.9	722.5
12	do	112.8	152.2	33.9	298.9	541.1	840.0	328.4	646.7	975.1
	Average	110.2	146.6	34.1	290.9	551.4	842.3	209.5	639.3	848.8

For three months the amount of calcium appearing in the drainage water collected from the field pots was practically the same for all of the sulphur treatments. This, however, should be expected, because up to this time no appreciable sulphofication had taken place. As the sulphur was changed to sulphate it might be expected that some of the sulphuric acid would react with the calcium in the soil. This is demonstrated by the amount of calcium appearing in the March and June drainage from the soils treated with sulphur. It is difficult to explain why the high sulphur treatments caused such small amounts of calcium to be present in the soil extracts. The determinations for the duplicate control soils show a wide variation which tend to throw a shadow of calcium in the leachings. This is especially true for the high sulphur additions. Under the conditions of the greenhouse experiment the data secured support the belief that sulphur rapidly depletes the supply of calcium in the soil. Further experimentation is needed, however, to confirm this statement for field conditions.

Working with various lime compounds, including calcium sulphate, Broughton [7] reached the conclusion that there is nothing in the soil to attract calcium in the form of the sulphate because $CaSO_4$ moves through the different depths in a solid column.

[7] BROUGHTON, L. B. HOW LIME IS DISTRIBUTED THROUGH AND LOST FROM SOILS. FACTORS INFLUENCING THE DIFFUSION AND DEPLETION OF LIME IN SOILS. Md. Agr. Exp. Sta. Bul. 166, p. 285-334. 1912.

If this were true, gypsum would not have any chemical action on the soil constituents. But it is evident from the discussion of the data obtained from experiment 2 that gypsum caused an increase in the amount of potassium in the leachings. Again, it is difficult to interpret the calcium data secured from the soils treated with gypsum in both experiments, because, first, of the wide variation in the calcium determinations in the water extracts from the two control soils in experiment 1 and, second, because of the wide difference between the duplicate gypsum treatments in the amount of calcium found in the leachings in the water-soluble magnesium in the soils at the close of the experiments.

Inasmuch as the differences between duplicate treatments in most cases are greater than the difference noted for the various sulphur treatments, it seems best to discuss only the total water-soluble magnesium data for the two experiments. Considering first the total water-soluble magnesium for experiment 1, it will be noted that none of the treatments had any appreciable effect on this element. The data from

TABLE VI.—*Amount of magnesium in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—magnesium in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—magnesium in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble magnesium	Leachings	Soil extracts	Total water-soluble magnesium
1	Control	15.0	10.7	45.0	70.7	205.2	275.9	19.2	Lost.	
2	do	13.4	5.9	20.6	39.9	189.6	229.5	23.5	187.4	208.7
	Average	14.2	8.3	32.8	55.3	197.4	252.7	21.3	187.4	208.7
3	Uninoculated sulphur 186	13.7	13.2	15.7	42.6	161.8	204.4	34.9	104.8	139.7
4	do	13.3.	10.1	23.9	47.3	182.6	229.9	50.3	167.1	217.4
	Average	13.5	11.6	19.8	44.9	172.2	217.1	42.6	135.9	178.5
5	Inoculated sulphur 189	14.0	5.6	19.3	38.9	168.7	207.6	52.5	174.9	227.4
6	do	13.4.	6.8	27.2	47.4	167.0	214.4	31.7	155.9	187.6
	Average	13.7	6.2	23.2	43.1	167.8	211.0	42.1	165.4	207.5
7	Uninoculated sulphur 1000	14.5	7.3	36.6	58.4	172.2	230.6	128.6	156.6	285.2
8	do	14.1	6.7	34.2	55.0	142.6	197.6	122.5	130.4	252.9
	Average	14.3	7.0	35.4	56.7	157.4	214.1	125.5	143.5	269.0
9	Inoculated sulphur 1015	19.1	8.8	45.7	73.6	93.9	167.5	136.7	137.6	274.3
10	do	14.7	10.2	35.4	60.3	168.7	229.0	132.1	123.8	255.9
	Average	16.9	9.5	40.5	66.9	131.3	198.2	134.4	130.7	265.1
11	Gypsum 1000	31.9	39.5	10.9	82.3	158.3	240.6	26.8	148.0	174.8
12	do	32.3	44.5	10.7	87.5	147.8	235.3	47.2	116.6	163.8
	Average	32.1	42.0	10.8	84.9	153.0	237.9	37.0	132.3	169.3

experiment 2. The results do indicate, however, that at least the greater portion of the calcium in the gypsum is leached away in the drainage water.

EFFECT OF SULPHUR AND GYPSUM ON MAGNESIUM

In Table VI appear the data showing the losses of magnesium in the drainage water and leachings and experiment 2 show that, while the low sulphur additions did not increase the amount of soluble magnesium, the high sulphur treatments did cause an increase in the amount of magnesium going into solution. In this case some of the soil magnesium was probably used to neutralize some of the acid which was produced in the oxidation of the 1,000 lbs. of elemental sulphur. Gypsum apparently had little effect in liberating magnesium from its insoluble compounds in the soil.

EFFECT OF SULPHUR AND GYPSUM ON SOIL NITROGEN

The data showing the effect of sulphur and gypsum on nitrogen in the soil and on nitrification are found in Table VII.

It should be remembered that the nitrogen which leached through the soils during December represents the accumulation of nitrates during the summer and fall of 1922, for there had been no rains sufficient to leach the soil previous to this time. No effect was noticed for the treatments during December. All of the remaining data indicate that the different treatments caused a slight depressing effect on nitrification. It is difficult to account for the relatively small amount of nitrates present in the leachings and water extracts from the soils in experiment 2. Possibly it was due to the utilization of nitrates by the nitrogen-assimilating organisms, or to the extremely small quantity of water-soluble phosphorus contained in this soil. McHargue and Peter[8] in their study of the removal of mineral plant food by natural drainage waters found that a high phosphorus content of soils accelerates the action of the nitrifying organisms.

EFFECT OF SULPHUR AND GYPSUM ON PHOSPHORUS

Analyses were made for phosphorus in the drainage water and leachings obtained from the soils in both experiments, but the amounts obtained were so small as to be well within the limit of error for this determination. These results are therefore omitted from the discussion. Most soils have shown the capacity to retain phosphorus compounds in the surface area, and usually only traces appear in the drainage.

TABLE VII.—*Amount of nitrogen in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—nitrogen in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—nitrogen in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble nitrogen	Leachings	Soil extracts	Total water-soluble nitrogen
1	Control	48.7	29.2	29.2	107.1	79.2	186.3	24.8	8.4	33.2
2	do	57.1	12.0	66.6	135.7	88.5	224.2	27.8	9.8	37.6
	Average	52.9	20.6	47.9	121.4	83.8	205.2	26.3	9.1	35.4
3	Uninoculated sulphur 186	58.3	18.6	14.6	91.5	55.9	147.4	21.1	8.1	29.2
4	do	54.5	15.0	15.0	84.5	51.2	135.7	23.7	7.3	31.0
	Average	56.4	16.8	14.8	88.0	53.5	141.5	22.4	7.7	30.1
5	Inoculated sulphur 189	50.3	15.9	16.0	91.2	60.5	151.7	17.3	7.0	24.3
6	do	51.1	15.1	16.2	82.4	55.9	138.3	21.6	5.8	27.4
	Average	55.2	15.5	16.1	86.8	58.2	145.0	19.4	6.4	25.8
7	Uninoculated sulphur 1000	58.6	17.8	11.6	88.0	51.2	139.2	18.0	7.8	25.8
8	do	56.0	13.6	18.6	88.2	55.9	144.1	24.2	8.4	32.6
	Average	57.3	15.7	15.1	88.1	53.5	141.6	21.1	8.1	29.2
9	Inoculated sulphur 1015	77.6	14.9	35.5	128.0	69.8	197.8	15.7	.....	.....
10	do	59.7	19.7	28.5	107.9	69.8	177.7	17.5	6.7	24.2
	Average	68.6	17.3	32.0	117.9	69.8	187.7	16.6	6.7	23.3
11	Gypsum 1000	52.2	15.3	12.7	80.2	55.9	136.1	16.8	5.0	21.8
12	do	57.1	16.1	13.3	86.5	55.9	142.4	24.3	6.7	31.0
	Average	54.6	15.7	13.0	83.3	55.9	139.2	20.5	5.8	26.4

[8] MCHARGUE, J. S., and PETER, A. M. THE REMOVAL OF MINERAL PLANT-FOOD BY NATURAL DRAINAGE WATERS. Ky. Agr. Exp. Sta. Bul. 237, p. 331-362, illus. 1921.

EFFECT OF SULPHUR AND GYPSUM ON HYDROGEN-ION CONCENTRATION

Table VIII gives the results of the determinations of the hydrogen-ion concentration in the samples of drainage water obtained from the pots in experiment 1 during the month of June, and in the leached soils at the close of the experiments.

Before the treatments were made the soil used in these experiments had a P_H of 7. The range of experimental error for the colorimetric method of determining hydrogen-ion concentration is usually considered to be 0.2 P_H. By taking this error into consideration and examining the determinations given for the duplicate treatments, it is noted that in every case at least one of these determinations falls within the 0.2 P_H limit. Therefore it can not be definitely said that any of the treatments increased the hydrogen-ion concentration in this soil or in the drainage water obtained during the month of June. It is surprising that considerable acidity did not develop in those soils receiving large additions of sulphur. A possible explanation for this fact is the high reserve supply of basic materials in the Palouse silt loam. As sulphuric acid was produced it was immediately neutralized by the bases present. Owing to its large supply of basic materials, this soil may receive considerable quantities of sulphur without showing the need of limestone to correct acid conditions.

The data given in Table VIII show also that there was insufficient acidity developed from sulphofication to bring about any solvent action on the native soil potassium and that the increase of this element, owing to the various treatments noted in Table IV, probably resulted indirectly from the formation of salts.

DISCUSSION OF THE CURVES

Each of the tables from III to VII, inclusive, gives a column representing the total water-soluble elements which were recovered in both experiments. The data giving the average of the duplicate treatments have been used

TABLE VIII.—*Hydrogen-ion concentration in the drainage water for June and in the soils at the end of the experiments*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)		Experiment 2 (green house pots)
		Drainage water for June	Soil extracts	Soil extracts
		P_H	P_H	P_H
1	Control	6.9	6.9	6.9
2	do	6.9	7.3	7.1
	Average	6.9	7.1	7.0
3	Uninoculated sulphur 186	6.7	7.2	6.7
4	do	6.7	7.3	6.5
	Average	6.7	7.1	6.6
5	Inoculated sulphur 189	6.6	7.4	6.7
6	do	6.7	7.0	6.9
	Average	6.65	7.2	6.8
7	Uninoculated sulphur 1000	6.7	7.1	6.7
8	do	4.6	6.9	6.6
	Average	5.65	7.0	6.65
9	Inoculated sulphur 1015	6.5	7.1	6.7
10	do	6.7	6.9	6.7
	Average	6.6	7.0	6.7
11	Gypsum 1000	6.7	7.4	7.3
12	do	6.8	7.2	6.9
	Average	6.75	7.3	7.1

in plotting the curves shown in Figure 1. The graph permits a comparison of the results obtained from both experiments, and also shows the relation of sulphofication to the availability of the soil potassium, magnesium, calcium, and nitrogen.

By examining the curves representing the different elements, and comparing the inoculated sulphur with the uninoculated sulphur, it is seen that, in general, the line connecting the two sulphur treatments is nearly always parallel to the abscissa. Especially is this true for the small applications of sulphur. It seems well established, therefore, that the uninoculated sulphur produced about the same effects in this soil as did the inoculated sulphur.

The curve representing the water-soluble calcium from the greenhouse pots follows the same general conformity as the sulphur curve for the greenhouse soils. This shows that with increased sulphofication there was a corresponding increase in the loss of calcium from the soils receiving sulphur, and this loss was greater with the high applications of sulphur than with the low sulphur treatments. The calcium curve for the field pots is apparently abnormal, or contrary to what would be expected to follow from the application of sulphur to the soil. The potassium curves show that increased sulphofication was followed by a greater availability of the soil potassium. Gypsum caused no marked effect on the water-soluble potassium, except under greenhouse conditions.

The curves representing the water-soluble magnesium obtained from the soils in both experiments show that the sulphur treatments had little effect on the loss of magnesium in the drainage water, except in the case of the large applications made on the greenhouse soils. The nitrogen curves likewise show that the applications of sulphur and gypsum had little effect upon nitrification.

CONCLUSIONS

From the data secured from this work on the Palouse silt loam, and presented in the foregoing tables and in Figure 1 (see p. 462), the following conclusions seem to be justified:

(1) The Palouse silt loam has a naturally high sulphofying power, and uninoculated sulphur when added to this soil was just as efficient as inoculated sulphur in producing sulphates.

(2) Under the conditions of the experiments the gypsum and sulphur used as fertilizers were readily leached out during the winter months. To guard against unnecessary losses of these materials it is advisable to apply them at a time when the crop has the greatest need for them.

(3) All of the sulphur treatments increased the availability of the native soil potassium, as is evidenced by the increased amount of this element in the drainage water from the soils which were treated with sulphur. Gypsum increased the amount of soluble potassium under greenhouse conditions, but definite increases in soluble potassium were not obtained under field conditions.

(4) Elemental sulphur, when oxidized in the soil under greenhouse conditions increased the loss of calcium in the leachings. The results on calcium obtained under field conditions were too irregular to point to any conclusions.

(5) Magnesium compounds in the soil were but little affected by sulphur or gypsum.

(6) Both the sulphur and gypsum treatments had little effect upon nitrification in this soil.

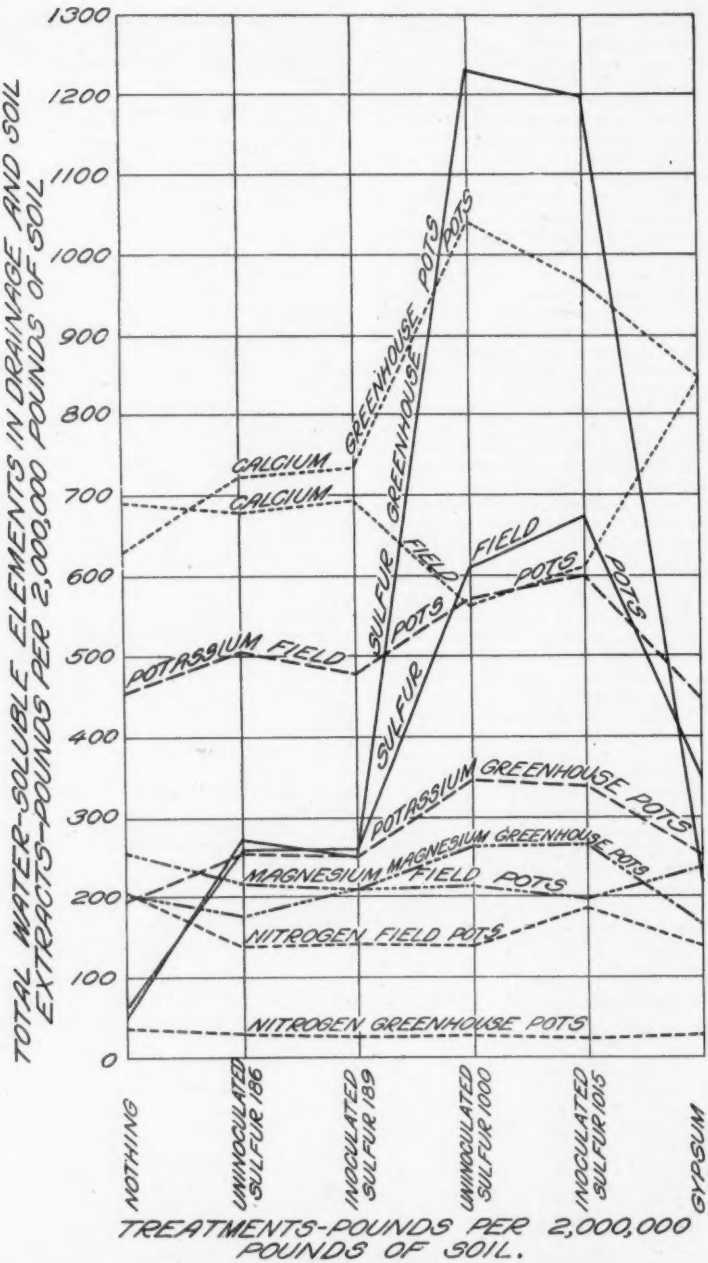


FIG. 1.—Effect of sulphur and gypsum on Palouse silt loam

PHYTOPHTHORA ROT OF PEARS AND APPLES[1]

By DEAN H. ROSE, *Pathologist*, and CARL C. LINDEGREN, *Field Assistant, Office of Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

On September 5, 1923, the junior author collected on the South Water Street Market, Chicago, Bartlett pears from Fennville, Mich., which showed black spots covering about half the pear. On the surface of these spots there was a scanty growth of mycelium (pl. 1, A). When the pears were cut through at these spots the flesh underneath was found to be for the most part very light brown in color but markedly darker brown in the vascular system (pl. 1, B). Mounts of the surface fungous growth under the microscope showed numerous conidia resembling those of Phytophthora, and plantings from the diseased flesh yielded a fungus which agreed in all important respects with published descriptions of *Phytophthora cactorum*. The pathogenicity of the fungus isolated from these pears and from apples and other pears collected later has been proved by inoculation into healthy pears and apples.

Further examination of fruits on the Chicago market showed that the disease is more widely distributed in the United States than had hitherto been suspected; a study of the fungus in culture and on the fruit brought out a number of facts concerning its morphology which seem not to have been observed by other workers. The present paper summarizes the results of this work and gives in addition a fuller description of the disease than is available in earlier publications.

DISTRIBUTION AND FIELD OBSERVATIONS

Following the collection of Phytophthora rot on the Chicago market on September 5, other collections were made as follows:

On September 11 a shipment of Clairgeau pears from St. Joseph, Mich., was seen on the Chicago market which showed a loss of about 30 per cent from a rot similar to that described above. It was learned later that the entire shipment consisted of windfalls.

On September 24 Anjou pears and on September 25 Clairgeau pears were found on the Chicago market which showed the symptoms of Phytophthora rot. Nothing was learned as to the origin of either of these lots of pears. It is probable, however, that they also came from Michigan, since they had been shipped in bushel baskets and Michigan was at that time the only Middle Western State shipping Anjou and Clairgeau pears in baskets into Chicago. Both varieties, if shipped from California or the Northwest, would have been packed in boxes.

On September 27 several orchards near St. Joseph, Mich., were visited and numerous cases of Phytophthora rot were found, on both pears and apples. The decay was found only on windfalls, never on fruit hanging on the tree. In some instances the windfalls under Clairgeau pear trees showed 100 per cent infection, particularly those under trees where there were indications that water had been standing on the soil. Moreover, pears which looked sound when viewed from above as they lay on the ground were usually found to have a black spot on the lower side if the ground was moist to the touch.

The varieties of pears found to be affected with Phytophthora rot were Clairgeau, Kieffer, Bosc, Howell, Bartlett, Anjou, and Angouleme (Duchess). The disease was also found to be serious among the windfalls on the following varieties of apples: Twenty-Ounce Pippin, Northern Spy, Tolman, Baldwin, Winesap, Jersey Sweet, Wealthy, Gideon, Rhode Island Greening, and Tompkins King. Cultures of a Phytophthora were obtained from all the varieties and all the collections mentioned above.

The influence of soil moisture was noted in two Kieffer pear orchards, both of which had been kept in clean culture. In one of these the soil was moist to the touch and practically all of the fruit on the ground showed the typical symptoms of Phytophthora rot. In the other orchard the surface soil

[1] Received for publication June 16, 1924; issued, May, 1925.

was almost dusty and only about 10 per cent of the windfalls showed the disease.

On October 29, 1923, the junior writer assisted in the inspection of a car of Delicious apples at Chicago, shipped from Wenatchee, Wash. Many of the apples were incrusted with clay, and one of them showed the characteristic symptoms of Phytophthora rot. A pure culture of Phytophthora was obtained from this apple. On January 11, 1924, the rot was found in Rome Beauty apples from Boise, Idaho, inspected in cold storage at Chicago. Plantings from affected fruits yielded only Phytophthora.

DESCRIPTION OF THE DISEASE

A brief description of Phytophthora rot on pears has been given in the first paragraph of this paper. However, there are certain characteristics of the rot on both pears and apples which merit further consideration, since a search of the literature shows them not to have been described in any detail before. Throughout the following discussion, which is based on a study of naturally infected fruits and of fruits inoculated in the laboratory, it is to be understood that all statements refer to tissues affected by the fungus. In both pears and apples there is always a marked vascular browning, both in the larger bundles near the core and in the smaller ones throughout the flesh (pl. 1, B and D). Very often the browning in the larger bundles extends to the stem and into it for part or all of its length (pl. 1, B) in the manner described by Schoevers (*12, p. 154*).[2] In this connection it may be mentioned also that Rose (*10*) found marked vascular browning in strawberries parasitized by a fungus very similar to and apparently identical with *Phytophthora cactorum*. In apples the flesh surrounding the bundles is lightly browned, in pears scarcely at all. In some varieties of pears, notably Clairgeau, the flesh is in fact decolorized and has a clear, water-soaked appearance very much like that of apple flesh affected by water-core. The parasitized flesh of both apples and pears sometimes becomes slightly spongy, but is usually as firm as sound healthy flesh; it rarely becomes soft and mushy like that found in lesions produced by Rhizopus or Penicillium. The only exception noted was that of pears of the Seckel variety, inoculated with Phytophthora in the laboratory. In cross-section, Phytophthora lesions on both pears and apples show indefinite boundaries so that it is impossible to make a clean separation of diseased from healthy flesh, as can so easily be done in fruits attacked by Rhizopus or Penicillium. The affected flesh has no marked odor or taste. Externally the lesions produced by Phytophthora on apples are light brown (pl. 1, C), on pears dark brown to black (pl. 1, A).

INOCULATION EXPERIMENTS

EXPERIMENT 1

Twelve Clairgeau pears were washed in soap and water and rinsed in sterilized tap water. They were then sterilized for 10 minutes in 1: 1,000 mercuric chloride solution, rinsed again in sterilized tap water, and placed in two moist chambers. Six were inoculated with a pure culture of Phytophthora obtained from Clairgeau pears and six were used as controls.

On the fourth day all the inoculated pears showed the characteristic lesions, and all the controls were free from any symptoms of the disease. The lesions ranged in size from 33 to 44 mm. in superficial diameter, averaging 37 mm. On the tenth day the controls were still in perfect condition, while the lesions on the inoculated fruits varied from 70 to 77 mm. in superficial diameter, averaging 74 mm. Cultures from these lesions yielded only Phytophthora.

EXPERIMENT 2

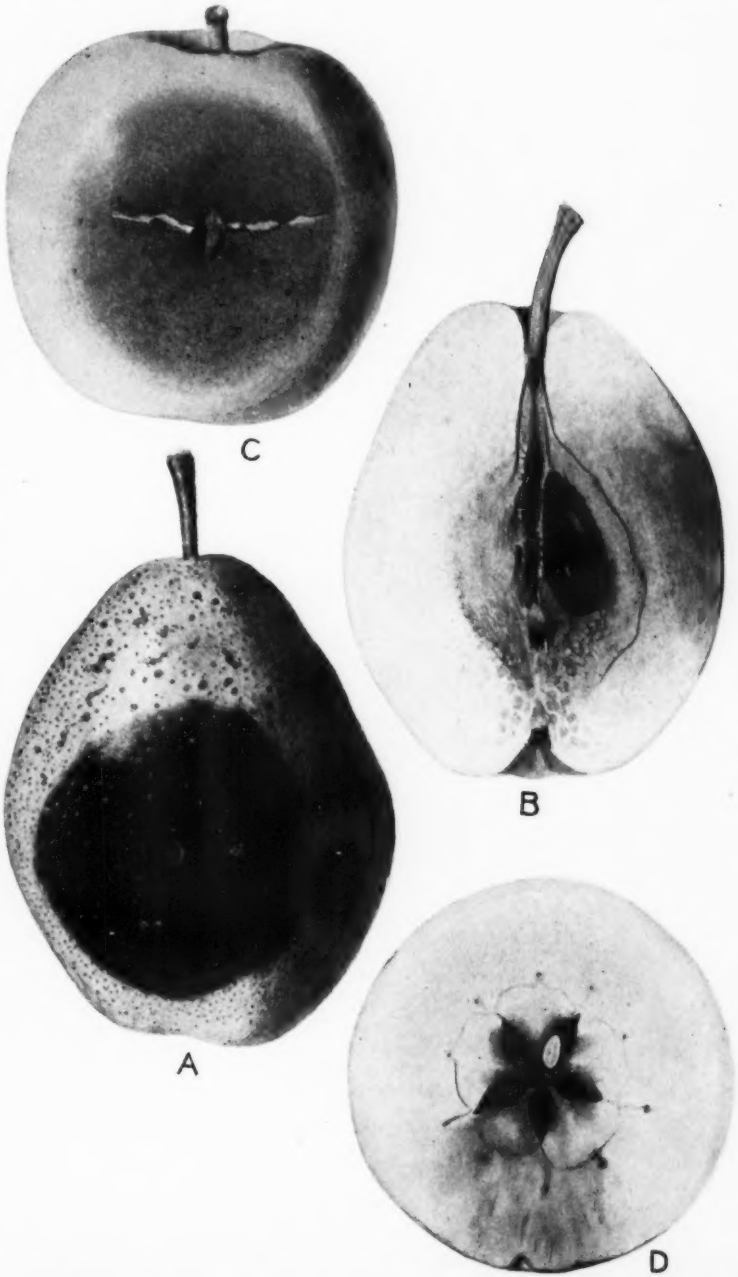
Eighteen apparently uninjured Kieffer pears were sterilized in 1: 1,000 mercuric chloride for 10 minutes. Six used for controls were placed in a moist chamber with water in the bottom.

A layer of cotton batting was placed in the bottom of a moist chamber and saturated with sterile water. On the cotton batting were placed six small pieces of an agar culture of the Phytophthora which had been isolated from Clairgeau pears. A sterilized pear was placed on each piece of agar culture. On the eighth day three of the pears, and on the thirteenth day all of them,

[2] Reference is made by numbers (italic) to "Literature cited," p. 468.

EXPLANATORY LEGEND FOR PLATE 1

A.—Anjou pear five days after inoculation with the Phytophthora isolated from a naturally infected pear
B.—Cross-section of the pear shown in A
C.—Grimes Golden apple five days after inoculation with the Phytophthora isolated from a naturally infected pear
D.—Cross-section of the apple shown in A (Color plate by J. Marion Shull)


C
B
A
D

showed the characteristic symptoms of Phytophthora. The fungus was recovered from two of these pears by culturing. All of the others were cut up and were found to show the characteristic vascular darkening.

EXPERIMENT 3

Some of the clay soil from an orchard at St. Joseph, Mich., was placed in a moist chamber and sterile water was poured over it until water stood on the soil to the depth of about 2 cm. Six sterilized pears, apparently free of skin punctures, were then placed in the moist chamber on the soil. All of these pears developed Phytophthora rot, and pure cultures of the organism were obtained from every one of them. The first pear developed the rot on the thirteenth day, two more on the twenty-first day, and the remaining three on the twenty-second day. Similar results were obtained in another experiment which duplicated No. 3, except that apples were used instead of pears. Controls provided in both experiments remained sterile.

Nothing is known of how infection occurred in the moist chambers containing pears resting on soil covered by water. Quite evidently the fungus existed in the soil and continued to grow there even though entirely submerged. It is possible and indeed quite probable that the fungus grew out into the water; but whether the infection which occurred was brought about by zoospores or directly by the mycelium is a question to which the experiments and the observations made on them give no answer.

The results obtained in these experiments are strong evidence that the Phytophthora isolated from apples and pears is able to penetrate the uninjured skin of those fruits. Evidence to the same effect was seen in the orchards visited in Michigan.

MORPHOLOGICAL FEATURES

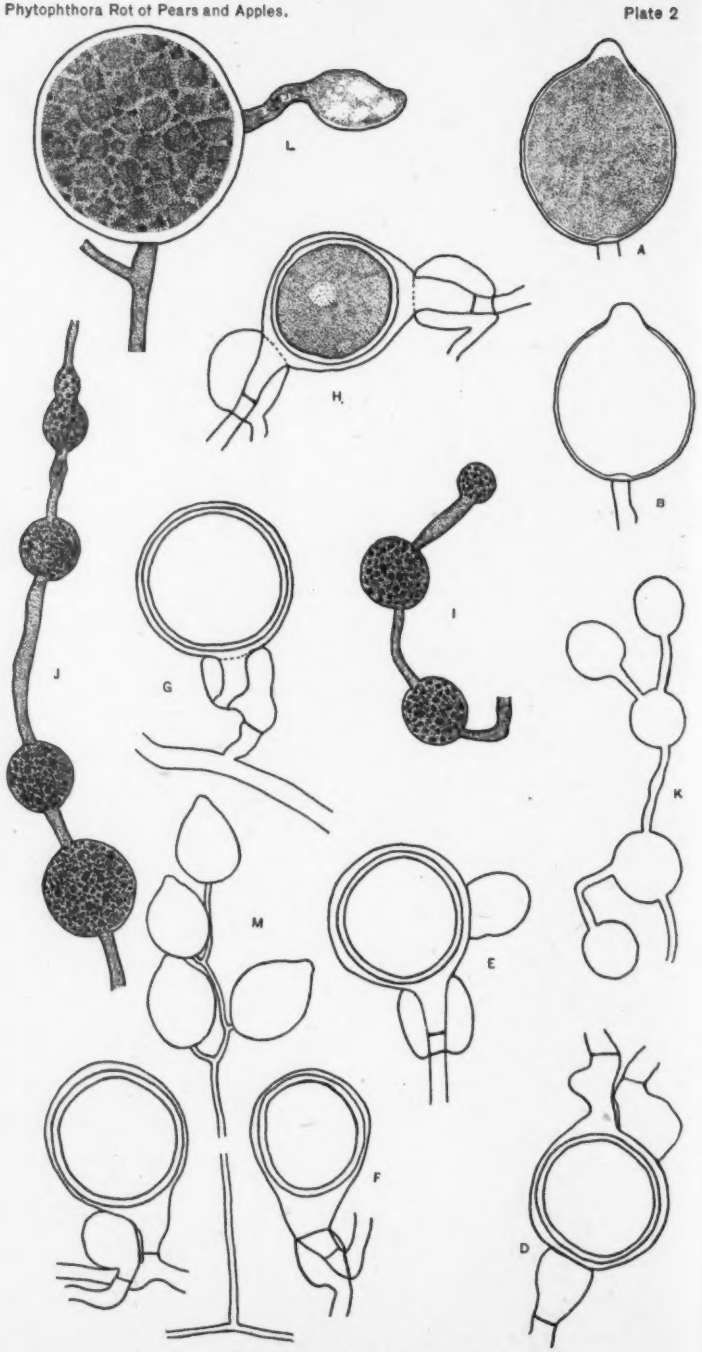
Twenty of the isolations from diseased pears and apples, including the three from Washington and Idaho apples, were subjected to comparative study after they had grown for 9 to 70 days on potato dextrose agar, on Sherbakoff's oatmeal agar, and on oatmeal paste. On potato dextrose agar they all produce conidia (pl. 2, A and B) and oogonia (pl. 2, C to H) in abundance and also large numbers of bodies which are very similar to the "sphaero-conidia" (pl. 2, I to L) described and figured for *Phytophthora cactorum* by Lafferty and Pethybridge (*6, p. 37 and pl. II, fig. 13*). On oatmeal agar they produced large numbers of conidia and oogonia, but very few "sphaero-conidia"; in some cultures there were none of these bodies. Measurements of the various spores forms were made for three cultures, which so far as could be told by examination with the microscope were representative of the whole 20. One of these cultures had been isolated from the Delicious apple from Washington, one from a pear from Michigan, and one from an apple from Michigan. The measurements can be summarized as follows: Oospores, 15 to 43 μ in diameter, average 26.6 μ; conidia, length, 25 to 44 μ, average 34.4 μ; conidia, width 21 to 31 μ, average 26.8 μ; "sphaero-conidia," 25 to 45 μ in diameter, average 34.6 μ. All of these are in close agreement with the constants given for the spore forms of *Phytophthora cactorum* by other workers. (Rosenbaum (*11*) Beach (*1*) and measurements quoted by the latter from other papers.)

The conidiophores of this fungus have been found as (1) short branches of the mycelium bearing a single conidium, or (2) longer branches bearing conidia in groups of 2 to 10 or more (pl. 2, M). The largest number of conidia seen in a single group was 15. Both kinds of branches arise from the mycelium in the manner shown for one of the longer branches, in the lower portion of Plate 2, M.

All three of the spore forms mentioned above were found on the surface of both pears and apples collected on the market. They also appeared on the surface of pears and apples inoculated in the laboratory, usually within six or eight days from the time of inoculation. Favorable conditions for this external growth seemed to be a moist atmosphere and a temperature of 20° to 25° C. or even slightly higher. A few oogonia were seen in mounts of diseased tissue from the inside of a number of fruits.

Measurements of the various spore forms as found on diseased fruits can be summarized as follows: Oospores 20 to 35 μ in diameter, average 26.6 μ; conidia, length 30 to 53 μ, average 40.3 μ; conidia, width 19 to 32 μ, average 36.9 μ; sphaero-conidia, for the few that were seen, 26 to 38 μ in diameter average 32.3 μ.

It will be seen that the conidia are slightly longer on the host than on culture media but that the other two spore forms average about the same size no matter where they are produced.



For explanatory legend see p. 467.

Antheridia are usually paragynous (pl. 2, C and D) but in old cultures on oatmeal paste a few have been found which were amphigynous (pl. 2, E, F, G, and H). Lafferty and Pethybridge (*6, p. 36*) have already reported this condition for cultures of *Phytophthora cactorum* obtained by them from various sources, including one furnished by H. H. Whetzel and said by him to have been isolated from a decayed apple grown in his own garden in Ithaca, N. Y. Rose (*10*) has also reported it for a Phytophthora isolated from decaying strawberries and shown by him to be very similar to *Phytophthora cactorum*. The writers wish to call attention, however, to an unusual form with two amphigynous antheridia shown in Plate 2, H. As seen under the microscope, the antheridia lay in approximately the same plane. It was thought at first that there were two oogonia, one lying over the other; but after repeated and vigorous tapping of the cover glass the object still remained as figured on Plate 2, one oogonium with two amphigynous antheridia.

The oogonium is apparently intercalary, but even so it is difficult to understand how it could have grown through two antheridia in the manner described by Pethybridge (*9*) for growth through one. In other words, the finding of the form here under discussion (pl. 2, H) raises the question whether for the apple and pear Phytophthora antheridia in the amphigynous position have that position because the oogonium has grown through them. The writers have no other explanation of how antheridia may become amphigynous but will merely add that at no time during a rather extensive study of the apple and pear Phytophthora have they seen early stages of the growth of the oogonium through the antheridium. Antheridia in the amphigynous position have been seen only in the final, fully developed stage and only in old cultures or in the older portions of cultures that were a month to six weeks old.

It should be added that an oogonium with two amphigynous antheridia has been seen only once. Attention is called, however, to Plate 2, D and E, which shows two oogonia, each with one amphigynous and one paragynous antheridium. One of these aggregates lay in an open space between two masses of mycelium and was made to assume different positions by pressure on the cover glass. Study of it in these positions showed that the structures figured in Plate 2, D, were not merely in fortuitous juxtaposition but were actually parts of one whole. The other (pl. 2, E) lay in a matrix of agar and mycelium and was studied only in the position shown by the drawing. It would be possible of course for one antheridium to assume a position at the side of an oogonium after the latter had grown through another antheridium. The figures are included here merely on the chance that they may at some time help to explain the condition shown in Plate 2, H.

DISCUSSION

The finding of Phytophthora rot on fruits on the Chicago market is a new record, so far as the pathological work is concerned which is carried on in connection with the Food Products Inspection Service. That is, the rot has not heretofore been seen in the course of this work, or, if seen, has not been recognized. The finding of the rot on pears and apples from Michigan and on apples from Washington and Idaho is, so far as the writers are aware, a new record for each of those States, though not for the continent of North America. Whetzel and Rosenbaum (*15*) reported *Phytophthora cactorum* from New York on Duchess apples in 1916, Hesler (*14, p. 172*) from New York on apples in 1918, Güssow (*4*) from Canada on pears in 1919, Clinton (*3, p. 454*) from Connecticut on pears in 1919 and on apples in 1920 (*3, p. 406*), and Gardner (*5, p. 53*) from Indiana on apples in 1921. *Phytophthora cactorum* is a well-known parasite of pears and apples in Europe (Wormald, (*16*), Osterwalder (*8*), Lafferty and Pethybridge (*6*)

EXPLANATORY LEGEND FOR PLATE 2

Spore forms and sporophore of the Phytophthor isolated from apples and pears. (A, B, C, D, E, F, G, H, L × 785. J, K, I, M × 369)

A, B.—Conidia
C.—Oogonium with paragynous antheridium
D, E.—Oogonia, each with one paragynous and one amphigynous antheridium
F, G.—Oogonia, each with one amphigynous antheridium
H,—Oogonium with two amphigynous antheridia
I, J, K, L.—Various forms of Sphaero-conidia
M.—Conidiophore with conidia attached. Lower portion of the figure shows manner of origin from the mycelium

and other references cited below). The above account indicates that the rot of pears and apples caused by a Phytophthora is rather widely distributed in the United States. It is granted of course that the apples from Washington and Idaho are not known positively to have become infected with Phytophthora in the State where they originated. Nevertheless, in the absence of proof to the contrary, it is fair to assume that they did become infected there.

It is noteworthy that during this investigation Phytophthora rot was found in one lot, the storage lot of Rome Beauty apples from Idaho, approximately four months after the usual time for harvesting the variety named. Most of those who have studied this disease have found it either in fruit in the orchard or in fruit which had been taken from the orchard only a few days before. (Bubak (*2*), Clinton (*3*), Güssow (*4*), Hesler (*14*), Marchal (*7*), Schoevers (*12*), Unamuno (*13*), Whetzel and Rosenbaum (*15*), and Wormald (*16*).) The apples in which Lafferty and Pethybridge (*6*) found the rot had been picked on October 14 and were sent to them on November 20. The authors make the statement (*6, p. 29*) that it is not known whether infection occurred prior to gathering or in storage. Osterwalder (*8, p. 440*) reports that he did not see the fungus at any time on stored apples. From the context the natural inference is that he is referring to the rot.

What was thought to be *Phytophthora cactorum* was also reported in 1922 from Pennsylvania on growing apple fruits by Thurston (*14*).

SUMMARY

There has been found on the Chicago market in pears and apples from Michigan and in apples from Washington and Idaho a rot from which a species of Phytophthora has been isolated.

This Phytophthora when inoculated into healthy pears and apples reproduced the diseases and was easily recovered from the affected tissues.

Evidence is presented which indicates that the Phytophthora isolated from pears and apples is similar to and probably identical with *P. cactorum*.

The observations of Lafferty and Pethybridge (*6*) are confirmed, that the apple and pear Phytophthora produces "sphaero-conidia" and both paragynous and amphigynous antheridia.

LITERATURE CITED

(1) Beach, W. S.
1922. The crown rot of rhubarb caused by Phytophthora cactorum. Pa. Agr. Exp. Sta. Bul. 174, 28 p., illus.

(2) Bubak, F.
1910. Die Phytophthorafäule der Birnen in Böhmen. Ztschr. Pflanzenkrank. 20: 256–261, illus.

(3) Clinton, G. P.
1920. New or unusual plant injuries and diseases found in Connecticut, 1916–1919. Conn. Agr. Exp. Sta. Bul. 222: 397–482, illus.

(4) Güssow, H. T.
1920. Phytophthora cactorum (Leb. et Cohn) Schroeter. Phytopathology 10: 50.

(5) Haskell, R. J., and Wood, J. I.
1922. Diseases of fruit and nut crops in the United States in 1921. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Surv. Bul. Suppl. 20, 138 p., illus. [Mimeographed].

(6) Lafferty, H. A., and Pethybridge, G. H.
1922. On a phytophthora parasitic on apples which has both amphigynous and paragynous antheridia; and on allied species which show the same phenomenon. Sci. Proc. Roy. Dublin Soc. 17: 29–43, illus.

(7) Marchal, E.
1908. Sur une maladie nouvelle du poirier. Bul. Soc. Roy. Bot. Belg. 45: 343–344.

(8) Osterwalder, A.
1905. Die Phytophthorafäule beim Kernobst. Centbl. Bakt. (II) 15: 435–440.

(9) Pethybridge, G. H.
1913. On the rotting of potato tubers by a new species of phytophthora, having a method of sexual reproduction not hitherto described. Sci. Proc. Roy. Dublin Soc. 13: 529–565, illus.

(10) Rose, D. H.
1924. Leather rot of strawberries. Jour. Agr. Research 28: 357–376, illus.

(11) Rosenbaum, J.
1917. Studies of the genus phytophthora. Jour. Agr. Research 8: 233–276, illus.

(12) Schoevers, T. A. C.
1915. Het phytophthora-rot der pitvruchten. Tijdschr. Plantenziekten 21: 153–159.

(13) Unamuno, L. M.
1911. Los estragos de la phytophthora cactorum en las peras y ciruelas. (Abstract) Ztschr. Pflanzenkrank. 21: 379.

(14) United States Department of Agriculture, Bureau of Plant Industry, Plant Disease Survey:
1918. Apple. Phytophthora rot caused by Phytophthora cactorum. U. S. Dept. Agr., Bur. Plant Indus., 2: 168–190. [Mimeographed.]
1922. Adams, J. F. Diseases of fruit and nut crops in the United States in 1922, U. S. Dept. Agr., Bur. Plant Indus., Suppl. 23: 392, illus.

(15) Whetzel, H. H., and Rosenbaum, J.
1916. The phytophthora rot of apples. Phytopathology 6: 89–90.

(16) Wormald, H.
1920. A phytophthora rot of pears and apples. Ann. Appl. Biol. (1919/20) 6: 89–100), illus.

SOME PHYSICAL AND CHEMICAL PROPERTIES OF CAROTIN AND THE PREPARATION OF THE PURE PIGMENT [1]

By F. M. SCHERTZ

Associate Biochemist in Soil Fertility Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In a previous paper (*6*)[2] certain colorimetric and spectrophotometric data have been given for carotin. By using the graphs from these data the quantitative results presented herein have been obtained. The method of procedure for the preparation of the pure carotin used in obtaining the data for the graphs and the solubility determinations is given in detail in this paper.

In connection with the solubility and with the quantitative determinations, it is essential to know what effect the solvent has upon the stability of the pigment in solution. Consequently, the keeping qualities of solutions of carotin have been studied.

Some idea of the practical application of the data contained in this paper and in the previous one may be obtained by a brief examination of Palmer's book on "Carotinoids and Related Pigments." The data presented should be of especial value to those engaged in the manufacture and purification of vegetable oils, for the pigments present in the vegetable oils (*5*) are closely related in spectral transmissive properties to the carotinoids, and very likely they consist of carotin and xanthophyll. Parsons and Wilson (*4*) have shown in Plate 1, page 270, of their paper [3] the absorption curves for petroleum oils. These curves show very clearly that it is possible to grade the oils when the transmissivity of the oil in question is known.

SOLUBILITY OF CAROTIN

Absolute ether distilled over sodium, ethyl alcohol (99.7 to 100 per cent), and redistilled petroleum ether (benzin petroleum) which fractionates at 30° to 50° C., were the solvents used in the solubility tests. Impurities in the ether (*1*) affected the solubility of carotin so that it was necessary to allow the ether to stand over sodium for several days and then freshly distill just enough for use.[4]

All of the solubility tests reported were made in a constant-temperature water bath which was kept at 25° C. The time the solutions remained in the bath and the amount of stirring varied somewhat, but it is stated for each set of determinations.

Either crystals of carotin freshly prepared, as will be described later in this paper, or those which had been kept in absolute alcohol in a sealed vial after filtering off and washing with low-boiling petroleum ether were used. They were dissolved in carbon disulphide or chloroform, and low-boiling petroleum ether was added to this solution. Crystals were obtained from this solution by evaporating the solvents under reduced pressure. The crystals were collected on a hardened filter in a small Büchner funnel and quickly washed two or three times with small portions of low-boiling petroleum ether, after which they were dried for 15 to 30 minutes in a well-evacuated desiccator. The melting point was quickly taken and if found satisfactory (174°) a small portion of the crystals was placed in each of two carefully cleaned bottles, which were partially filled with their respective solvents—alcohol or petroleum ether—and placed in the water bath for three hours, during which time the bottles were frequently shaken.

In most cases 10 c. c. of the saturated solution were withdrawn from the bottle which had been kept in the water bath. By carefully tying a filter paper on the end of a pipette, 10 c. c. of the saturated solution of carotin could be

[1] Received for publication June 6, 1924; issued May, 1925. This paper is a report of an investigation on the four chloroplast pigments.

[2] Reference is made by number (italic) to "Literature cited," p. 474.

[3] Spectrophotometric methods would undoubtedly be far more accurate than the method which they describe, and in addition would be simpler. The fact that all of the paraffin base oils (light and dark) have essentially the same light absorption curve makes the spectrophotometric methods even more useful in determining the grade of the oil.

[4] The impurities probably were peroxides, which may be removed by distilling the ether over sodium.

withdrawn without removing any of the crystals. The saturated alcoholic solution as a rule was made up to a volume of 50 c. c., or diluted 5 times; the saturated petroleum ether solution was diluted 100 or 200 times, and the saturated ether solution was diluted 1,000 times for the spectrophotometric determinations. In every case U. S. P. ether was used in making the dilutions, which were all made at room temperature a few minutes before the spectrophotometric readings were taken in a 2 cm. cell. The scale on the spectrophotometer was set so as to use the mercury line 435.8 mμ, in which position readings were made.

The quantity of pigment in the various solutions was determined at room temperature by means of a König-Martens spectrophotometer in the manner described in a previous paper (*6*).

Experiments 3, 4, 5, 6, 7, and 8 of Table I were made in a slightly different way than 1 and 2. In these cases the solubility tests were made in 30 c. c. bottles placed inside a fruit jar which was closed tightly so that no water might enter. The jar and its contents were then mechanically revolved in the water bath for the time specified.

In experiments 6, 7, and 8 the carotin crystals used were taken from the same preparation of carotin, the other experiments being on different samples.

TABLE I.—*Solubility of carotin at 25° C. in absolute alcohol*

Experiment No.	Dilution	Approximate time in water bath	Transmittancy	Carotin per liter
	Times	*Hours*		*Mgm.*
1	5	3	0.2170	8.3
2	5	12	.1700	9.6
3	5	15	.1920	9.0
4	5	20	.0673	14.8
5	5	20	.0572	15.6
6	5	48	.0718	14.3
7	5	72	.0671	18.0
8	5	90	.0693	14.6

It appears that after 20 hours saturation was reached in the alcohol solution; and, by averaging all of the figures for the solubility at 20 to 90 hours in the water bath, the solubility for carotin in absolute alcohol is 15.5 mgm. per liter at 25° C.

By averaging the results for 48, 72, and 90 hours in Table II, it is found that about 626 mgm. of carotin dissolved in one liter of petroleum ether at 25° C.

TABLE II.—*Solubility of carotin at 25° C. in petroleum ether, B. P. 30° to 50°*

Experiment No.	Dilution	Approximate time in water bath	Transmittancy	Carotin per liter
	Times	*Hours*		*Mgm.*
1	100	3	0.0610	305
2	100	15	.0310	382
3	100	20	.0200	428
4	200	20	.0890	526
5	200	48	.0634	604
6	200	72	.0724	570
7	200	90	.0490	704

The solubility in ether was obtained with difficulty, on account of the instability of carotin in ether solutions, as already mentioned.

Table III shows the solubility of carotin in specially purified ether. At 30 hours the equilibrium seemingly has become established and the average results show that approximately 1,005 mgm. of carotin are soluble in a liter of ether at 25° C.

TABLE III.—*Solubility of carotin at 25° C. in absolute ether*

Sample No.	Dilution	Approximate time in water bath	Transmittancy	Carotin per liter
	Times	*Hours*		*Mgm.*
1	1,000	30	0.402	980
2	1,000	46	.377	1,050
3	1,000	54	.398	1,010
4	1,000	90	.402	980

Boiling ether was found by Willstätter (*8*) to dissolve about 1.11 gm. of carotin per liter and low-boiling petroleum ether on a reflux condenser to dissolve 0.66 gm. per liter, while he observed that carotin was very difficultly soluble in absolute alcohol.

STABILITY OF CAROTIN IN SOLUTION

A small quantity of pure carotin was placed in each of three flasks; absolute alcohol was added to one, to another absolute ether (not freshly distilled), and to the third petroleum ether (30° to 50°). The solvents used were of the same grade as those employed in the solubility tests above, except that the ether was taken directly from the container in which it came.

These solutions were stored in the ice box and from time to time the transmittancy was determined spectrophotometrically. Dilution was often found necessary, because the instrument is best adapted for use with carotin solutions of low concentration.

In Table IV are presented results obtained with solutions of carotin in alcohol, in ether, and in petroleum ether, when kept for varying lengths of time.

TABLE IV.—*Spectrophotometric determinations showing the stability of carotin in alcohol, in ether, and in petroleum ether when stored in the ice box for varying lengths of time*

Number of days	Transmittancy	Solution	Dilution	Carotin per liter
			Times	*Mgm.*
0	0.0403	Absolute alcohol	×1	3.64
	.1360	Absolute ether	×20	43.60
	.0076	Petroleum ether	×20	50.80
24	.0424	Absolute alcohol	×1	3.58
	.0991	Absolute ether	×4	10.12
	.0149	Petroleum ether	×10	46.00
38	.0386	Absolute alcohol	×1	3.68
	.0557	Absolute ether	×2	6.32
	.1050	Petroleum ether	×20	49.00
50	.0408	Absolute alcohol	×1	3.63
	.0513	Absolute ether	×1	3.24
	.1047	Petroleum ether	×20	49.00
71	.0420	Absolute alcohol	×1	3.60
	.1510	Absolute ether	×1	2.05
	.0982	Petroleum ether	×20	50.40
111	.0439	Absolute alcohol	×1	3.55
	.3420	Absolute ether	×1	1.16
	.1020	Petroleum ether	×20	50.00
143	.0444	Absolute alcohol	×1	3.54
	.4610	Absolute ether	×1	.83
	.0906	Petroleum ether	×20	52.40

The results obtained in Table IV are graphically shown in Figure 1. Carotin apparently is just as stable in petroleum-ether solution as it is in alcohol solution. The rapid decline in the ether curve at once indicates that decomposition of the carotin in the ethereal solution has been proceeding during the course of the experiment. The oxidation of the carotin may be due to the formation of peroxides (*1*) in the ether, as already pointed out.

These facts are of importance to anyone who is interested in keeping carotin solutions for any purpose whatsoever. The data for the alcohol solution show that it is possible to make up a standard solution of pure carotin in absolute alcohol and keep it for months, during which time it may be used for comparative work. The facts as observed are also of importance in connection with the method of determining the amount of carotin in any substance—for example, in green leaves. In the method of separating the plant pigments, carotin is obtained in solution in petroleum ether. This solution of carotin in petroleum ether may be stored in the ice chest for several days without fear of decomposition before making spectrophotometric, colorimetric, or comparison determinations of the amount of carotin which it contains. This is a decided convenience if a great number of determinations are to be made with a colorimeter, for such determinations can not be made at all on some days, nor at any time during the day, because of light conditions.

SEPARATION AND PURIFICATION OF CAROTIN FROM CARROTS

Carotin may be obtained from carrots in the manner described below:

After careful washing to remove any adhering dirt or foreign material, the carrots are sliced in a power slicer or by hand, using a vegetable slicer; the knives should be sharp, so as to injure the tissue as little as possible. The cut pieces should be about 4 mm. thick. The sliced carrots are placed on a large galvanized-iron wire tray and dried in an oven in which the temperature is never allowed to exceed 50° C.

Since destruction of a large percentage of the carotin takes place during drying, the process should be carried on as rapidly as possible till the carrots are dry enough to grind in a power feed mill. The finely ground carrots will probably need to be dried further for several hours in a vacuum oven at less than 50° C. Grinding is rendered difficult if the material is not fully dry, as the presence of sugar in the carrots tends to clog the mill. After the final drying in the vacuum oven, the carrot meal is further ground in a ball mill (about 12 hours) till the greater part will pass a 40-mesh sieve. The finer it is ground, the more slowly the solvent will percolate and the higher will be the yield of the extracted pigment.

The pulverized carrots (3 kgm.) are placed in a glass percolator (10 cm. in diameter at the top and 60 cm. long) provided with a layer of cotton in the bottom to prevent the fine red powder from going through, and the percolator is then attached to a large suction filter flask. Pure redistilled petroleum ether (B. P. 30° to 60° C.) is added and suction is applied till the solvent just begins to run through. More petroleum ether is then added and allowed to percolate slowly overnight without using suction, when a large quantity of the colored extract will have passed

through into the flask. More petroleum ether is added and reduced pressure is applied till all of the colored extract has been removed. About 7 liters of petroleum ether are necessary to extract the 3 kgm. of dry powder which represent a little over 1 bushel of fresh carrots. From 1 bushel (50 pounds) of carrots, 2,774 gm. of dry carrot powder ready for extraction are obtained. In all, an afternoon and the following morning are required to complete this extraction.

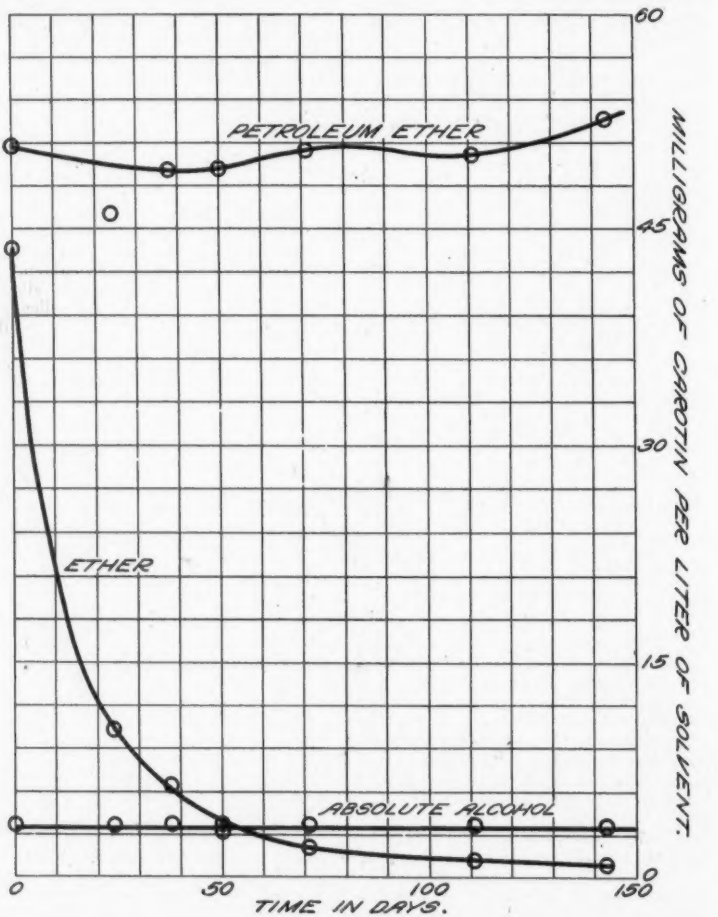


FIG. 1.—Stability of carotin in solution

The total red-brown percolate, from which crystals may be already separating, is now concentrated on a steam-heated water bath, the temperature of which is never allowed to exceed 50° C. About half of the petroleum ether used may be recovered by careful distillation. The concentrated petroleum ether solution is further concentrated under reduced pressure till most of the carotin has separated in the crytalline form. During the last stages of concentrating a fine current of carbon dioxide which has been led through a calcium chloride drying tube and then into the flask through a capillary is advantageous, because it prevents frothing over, aids evaporation, and also keeps the solution under an inert gas. In addition, glass beads are added to prevent bumping. After

concentrating to about 250 c. c., a stronger suction is applied so as to evaporate the petroleum ether and cool the solution in order that more of the carotin will crystallize; during this evaporation the flask is removed from the water bath to cool the solution. Or, after concentrating to 250 c. c., the concentrated carotin solution may be placed in an ice chest. In two or three days most of the carotin will have crystallized from the solution. The crystals are then filtered off.

After separating the gold, shimmering mass from the mother liquor by filtration, the crystals are dissolved on the same filter in the least possible quantity of chloroform or carbon disulphide, from which they are precipitated by shaking vigorously with small portions (50 to 100 c. c. in all) of absolute alcohol. Greatly reduced pressure may be used here, which will cause the chloroform or carbon disulphide to evaporate and cool the solution. Both the cooling and the evaporation of the solvent aid in crystal formation and cause a larger yield. After about one hour the crystals are filtered off either on a filter paper or a linen cloth; the flask in which the precipitation took place is rinsed with low-boiling petroleum ether (25 to 50 c. c.), and this is used to wash the crystals which are on the filter paper or cloth in the Büchner funnel. This washing will dissolve most of the remaining fats and waxes. The crystals are washed again with low-boiling petroleum ether and suction is applied till they are free of this solvent. Willstätter washes here with a half-and-half mixture of alcohol and petroleum ether; this mixture has the advantage that it does not dissolve as much of the crystals and the disadvantage that it does not dissolve the impurities as well as pure petroleum ether.

To avoid the possibility of the carotin crystals containing alcohol of crystallization (*7*) they are recrystallized by dissolving in just enough chloroform or carbon disulphide to effect solution and then adding petroleum ether (30° to 60°) and evaporating to about 25 c. c. or less. The crystals are collected on a hardened filter paper and washed two or three times with 10 to 15 c. c. portions of low-boiling petroleum ether. Any traces of fats, waxes, or oils should be removed by this last precipitation and final washing with petroleum ether. The pure pigment thus obtained is dried for 15 to 30 minutes in a good vacuum desiccator. The melting point is determined and, if found satisfactory (174°), the samples desired are rapidly weighed, placed in glass tubes filled with CO_2, sealed or dissolved for use, and the remaining crystals stored immediately, as described below.

Carotin crystals in mass are colored dark copper-red. When viewed through the microscope they have an orange-red color (*3*). The color and crystal form are illustrated by Escher (*2*) and the crystal form by Willstätter and Stoll (*8*). Carotin nearly always crystallizes in plates or leaves which often are rhombic or quadratic, and rarely does it crystallize in needles. The form and the size of the crystals vary with the solvent, the amount of the impurities in the solution, the temperature, and the time allowed for crystallization.

Very dilute solutions of carotin in alcohol, ether, or petroleum ether are yellow, more concentrated ones are deep orange, and very concentrated ones are deep red, except in the case of alcohol where the saturated solution is yellow. A weak solution of carbon disulphide is pink to red, whereas the more concentrated ones are red, dark red to almost black.

Escher (*2*) has obtained 125 gm. from 5,000 kgm. or 1 gm., of carotin from 40 kgm., (equivalent to 0.56 gms. per bushel) of fresh carrots, which is perhaps the best yield reported in the literature. On a small scale, Escher obtained from carrots which he himself dried, carotin at the rate of 0.10 gm. per kgm. of fresh carrots (equivalent to 2.27 gm. per bushel). He determined colorimetrically that carrots contained as much as 0.023 per cent of their fresh weight as carotin, or approximately 5.22 gm. per bushel, whereas the same variety when dry contained 0.013 per cent (based on fresh weight) of carotin or 2.95 gm. per bushel. Hence, there was a loss of 43.5 per cent of the carotin during the drying process.

In this investigation, 1 bushel (50 pounds) of fresh carrots contains from 1.68 to 3.46 gm. of coloring matter estimated colorimetrically as carotin. The same carrots when dried and ground in a ball mill ready for extraction contained respectively 1.41 to 2.81 gm. of carotin (based on a bushel of fresh carrots). From the bushel of carrots which contained 2.81 gm. of carotin when dry, 1.13 gm. of pure carotin, crystallized from petroleum ether and having a melting point of 174° C., were obtained. The loss in drying, 16.1 and 18.8 per cent, respectively, is probably explained by the manner of slicing the carrots.

Some fresh carrots were sliced and some were ground. Carotin decomposition was much greater in the ground carrots owing perhaps to the greater mechanical injury to the tissues in the grinding process, greater exposure of the material to oxygen, and slower drying. Incomplete extraction and losses in the crystallizations are further responsible for the low yield obtained.

SUMMARY

(1) The solubility of carotin at 25° C. in absolute alcohol was found to be 15.5 mgm. per liter; in petroleum ether (B. P. 30 to 50° C.), 626 mgm. per liter; in specially purified ethyl ether, 1,005 mgm. per liter.

(2) Solutions of carotin in absolute alcohol and petroleum ether were found to be extremely stable when kept in the ice box. An ether solution of carotin decomposed rapidly though stored in the ice box. The decomposition may have been caused by peroxides in the ether.

(3) Crystals of carotin may be stored in alcohol or petroleum ether for some time with only slight oxidation even though the container is not sealed, and may be stored permanently in these solvents if sealed in ampules.

(4) The method for the preparation of pure carotin from carrots on a small scale is described.

(5) A bushel of carrots (50 pounds) which contained 3.46 gm. of carotin colorimetrically when fresh, and 2.81 gm. of carotin colorimetrically when pulverized for extraction, yielded 1.13 gm. of pure carotin (M. P. 174°).

LITERATURE CITED

(1) CLOVER, A. M.
1922. THE AUTOXIDATION OF ETHYL ETHER. Jour. Amer. Chem. Soc. 44 : 1107–1118.

(2) ESCHER, H. H.
1909. ZUR KENNTNIS DES CAROTINS UND DES LYCOPINS. 102 p., illus. Zürich.

(3) KOHL, F. G.
1902. UNTERSUCHUNGEN ÜBER DAS CAROTIN UND SEINE PHYSIOLOGISCHE BEDEUTUNG IN DER PFLANZE. 206 p., illus. Leipzig.

(4) PARSONS, L. W., and WILSON, R. E.
1922. A NEW METHOD OF COLOR MEASUREMENT FOR OILS. Jour. Indus. and Engin. Chem. 14: 269–278, illus.

(5) PRIEST, I. G.
1920. THE COLOR OF SOYA BEAN OIL AS COMPARED WITH THAT OF COTTONSEED OIL. Cotton Oil Press 3(9): 37–40, illus.

(6) SCHERTZ, F. M.
1924. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26 : 383–400, illus.

(7) WILLSTÄTTER, R. M., and MIEG, W.
1907. UNTERSUCHUNGEN ÜBER CHLOROPHYLL. IV. UEBER DEN GELBEN BEGLEITEN DES CHLOROPHYLLS. Liebig's Ann. Chem. 355 : 1–28.

(8) ——— and STOLL, A.
1913. UNTERSUCHUNGEN ÜBER CHLOROPHYLL; METHODEN UND ERGEBNISSE. 424 p., illus. Berlin.

THE LIFE HISTORY OF THE TEXAS ROOT ROT FUNGUS, OZONIUM OMNIVORUM SHEAR [1]

By C. L. SHEAR

Senior Pathologist, Bureau of Plant Industry, United States Department of Agriculture

The fungus now generally accepted as the cause of root rot of cotton and a large number of other cultivated and wild plants in Texas and other parts of the Southwest as far as southern California was first described by Pammel [2] and tentatively referred to *Ozonium auricomum* Link. This fungus seems to be a native of the southwestern United States and is most common in the so-called "black waxy soils" of Texas; but it seems to be becoming more widespread and more serious in southwestern Arizona in recent years with the increased growing of alfalfa and cotton there.

Investigations of this fungus made by the writer in the period 1902 to 1907 and the comparison of it with Link's type of *O. auricomum* in Berlin indicated that the organism was quite different from Link's species, and as no description which seemed to apply to it could be found in the literature it was described and named *Ozonium omnivorum* Shear.[3]

At that time the fungus was known only as a sterile mycelium. Circumstantial evidence, however, based upon the occurrence of a conidial fungus producing spores in great abundance on the surface of the soil in portions of cotton fields where the plants had been killed by the fungus, suggested that this might be a sporogenous form of the Ozonium.

Duggar,[4] in 1916, reported the presence of conidia-bearing hyphae on the characteristic mycelium of the Ozonium found on the roots of affected plants, and also the identity in artificial culture of the mycelium obtained from diseased roots with that obtained from cultures of these conidia.

The conidial condition of the fungus (fig. 1) studied by Duggar was referred to the genus Phymatotrichum of the Hyphomycetes and the new combination *Phymatotrichum omnivorum* (Shear) Duggar proposed.

Taubenhaus and Killough [5] state that the senior author has confirmed Duggar's work by producing conidia of the Ozonium in pure culture on sterilized soil in the laboratory. Only one out of six cultures, however, produced conidia. As this spore form belongs to the so-called imperfect fungi which are believed to be merely conidial conditions of the higher forms, it was natural to suppose that a perfect stage of the fungus might still occur, though perhaps rarely.

In hope of determining the complete life history of the fungus, numerous cultures were made on different media and kept under various conditions for long periods, but they always remained sterile. Careful observation and search in diseased areas on cotton roots and stems which had been killed by the fungus were made at different seasons, especially in the winter, spring, and early summer. Numerous fungi were, of course, found but none could be proved to belong to the root-rot fungus. In August, 1903, however, the writer found what he believes to be the perfect stage of this Ozonium, although the evidence at present is circumstantial. In a cotton field near Paris, Tex., there was a so-called "dead spot" of cotton in which the plants had all been killed by this fungus, and immediately adjoining this spot was a hedge of osage orange (*Maclura aurantiaca* Nutt.). In the margin of this diseased area, not far from the dead and dying cotton plants, was a small sprout of the osage orange about 1 foot high which showed the characteristic wilt produced by the Ozonium. An

[1] Received for publication June 27, 1924; issued May, 1925.

[2] PAMMEL, L. H. COTTON ROOT-ROT. Tex. Agr. Exp. Sta. Ann. Rpt. (1889) 2 : 73, pl. 1-3. 1890.

[3] SHEAR, C. L. NEW SPECIES OF FUNGI. Bul. Torrey Bot. Club 34 : 305. 1907.

[4] DUGGAR, B. M. THE TEXAS ROOT ROT FUNGUS AND ITS CONIDIAL STAGE. Ann. Mo. Bot. Gard. 3 : 11-23 illus. 1916.

[5] TAUBENHAUS, J. J., and KILLOUGH, D. T. TEXAS ROOT ROT OF COTTON AND METHODS OF ITS CONTROL. Tex. Agr. Exp. Sta. Bul. 307, p. 5, illus. 1923.

examination of the wilting plant showed that the typical mycelial strands of yellowish hyphae of Ozonium extended from the soil up the stem several inches and there began to change into a subiculum which surrounded the stem and formed a hymenium with the typical spines of a species of Hydnum, as illustrated in the accompanying plate (pl. 1). The organic connection between the typical Ozonium hyphae and those of the Hydnum appeared to be unquestionable.

In order to complete the proof of the genetic relationship of the two forms, efforts were made to grow the fungus from the Hydnum. Unfortunately, however, all of our efforts in this direction failed.

Conidial forms similar to the Phymatotrichum stage of this fungus are known to occur among the Hymenomycetes and there is nothing in this life history which conflicts with our present knowledge of the subject.

The Hymenomycete, *Tomentella granulata*, according to Brefeld [6] has a conidal form very similar to that of *Ozonium omnivorum*. The Ozonium mycelium has a general similarity to the various sterile mycelia described by Persoon under the generic name Fibrillaria, some of which are known to belong to hymenomycetous fungi.

Being unable to find named specimens or a description to apply to this Hydnum, a description and illustration are given which it is hoped will make it possible for mycologists and pathologists who may encounter the fungus to recognize it.

Hydnum omnivorum n. sp.

Subiculum effuse, thin, readily separating from the substratum, subgelatinous, pale yellow, margin floccose, fibrillose-fimbriate, white; spines slender, acute, somewhat crowded, 2–3 mm. long, orange-buff to light orange-yellow (Ridgway). No spores found.

Conidial stage (*Phymatotrichum omnivorum*). Hyphae forming a loose layer on the surface of the soil in areas where the root rot has killed the plants; fertile hyphae arising irregularly from the mycelium, simple or forked; conidia numerous, sessile, borne on the irregularly swollen clavate or subglobose

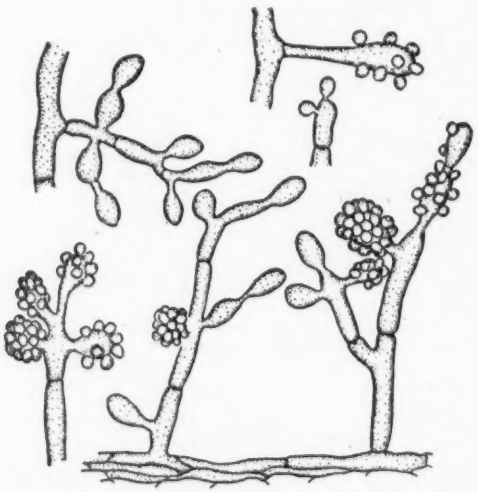


FIG. 1.—*Phymatotrichum omnivorum:* Conidial stage of the fungus, showing mode of conidial production (after Duggar)

[6] BREFELD, O. BASIDIOMYCETEN III. p. 11–12, tab. 1, fig. 16. 1889. (*In his* Untersuchungen aus dem Gesammtgebiete der Mykologie, Heft 8.)

EXPLANATORY LEGEND FOR PLATE 1

Life history of Ozonium omnivorum Shear
A.—Hyndum omnivorum. Natural size on wilting Maclura
B.—Portion of the fungus, with spines enlarged 20 diam.

A
B

short terminal or lateral branches of the hyphae or on the sides of the undifferentiated hyphae, pale ochraceous to gray in mass, appearing nearly colorless when separate, globose to ovoid 4 to 6×5 to 8 μ, smooth.

Sterile mycelial stage (*Ozonium omnivorum*) mycelium thin, floccose to arachnoid, forming thin branching strands, the dirty ochraceous branches arising from these strands are thin, somewhat rigid and branch at right-angles from somewhat swollen nodes, 2 to 4 branches from a node, usually long, thin, tapering and acute (fig. 2).

Type No. 5267 C. L. S. on wilting *Maclura aurantiaca* near Paris, Tex., September, 1903. In herbarium pathological collections, Bureau of Plant Industry, U. S. Department of Agriculture.

This plant has something of the general appearance of some of the yellow resupinate species of Hydnum which have been described, but appears to be distinct, especially in its parasitic habit and apparent restriction to the southwestern United States and Mexico, the only region where this root rot is known. The perfect stage probably occurs as a saprophyte also, as specimens very similar, if not identical, have been found on old over-wintered cotton stalks killed by the root rot.

Unfortunately the character of the basidiospores can not be given, as none could be found on the specimen described. The enlarged spines in the plate had the tips broken off in most instances. When whole they are acute, as indicated in a few cases. The color in fresh specimens is brighter than in the dried, as it fades in drying.

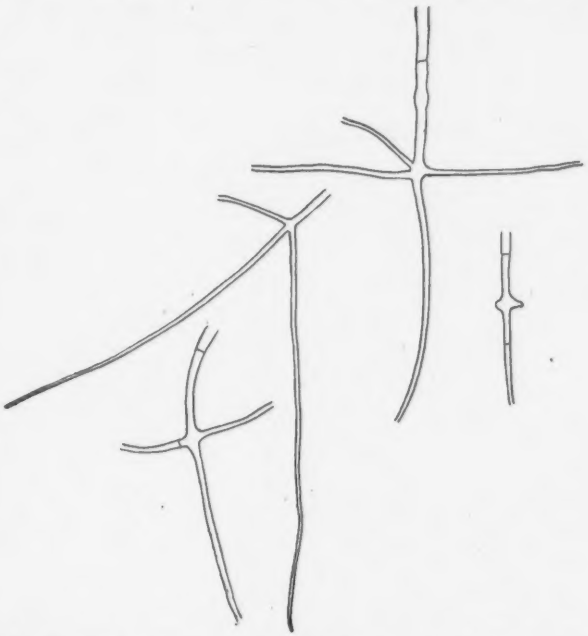


FIG. 2.—*Hydnum omnivorum:* Ozonium stage, showing characteristic modes of branching of the terminal hyphae. × 420

THE DISTRIBUTION OF THE ALFALFA WEEVIL (PHYTONOMUS POSTICUS GYLL. A STUDY IN PHYSICAL ECOLOGY[1]

By WILLIAM C. COOK,
Assistant Entomologist, Montana Agricultural Experiment Station

INTRODUCTION

The alfalfa weevil (*Phytonomus posticus* Gyllander) is a recently introduced insect which is doing a large amount of damage in the Great Basin region. It was first noted near Salt Lake City, Utah, in 1904, and has spread until up to the present time it has been captured in Oregon, Idaho, Wyoming, Colorado, Nevada, and California, as well as in Utah. In many parts of its present range it is capable of doing an immense amount of damage, at times almost completely destroying the alfalfa crop. Although the area now occupied by the insect is a small portion of the total area devoted to alfalfa growing in the United States, the insect is regarded with alarm by all of the neighboring States, which have enacted quarantine laws against alfalfa in an effort to retard the further dispersion of the weevil.

At the present time the insect is rapidly spreading into new territory, and it would be a matter of great interest and value to determine, with some degree of accuracy, the probably final limits of its distribution in America. This paper is an attempt to determine, from the climatic limitations found in Europe and Asia, the probable economic distribution of the alfalfa weevil in America. The study will be presented in three parts: (1) A compilation of all known information concerning the effects of climatic factors on the life history and abundance of this species in America; (2) a study of its distribution in Eurasia, to determine the optimum and limiting climatic conditions; and, (3) the application of this information to a study of climatic conditions in America, to locate the regions of optimum and limiting conditions, and to outline the distribution.

CLIMATIC ECOLOGY OF PHYTONOMUS POSTICUS IN AMERICA

The data which are quoted in this portion of the paper have been taken from various papers on the alfalfa weevil, and the material has been quoted in full in each case, following the authority, which is placed in parentheses.

OUTLINE OF LIFE HISTORY

The alfalfa weevil winters in Utah as an adult in trash and weeds in fields. Early in the spring the adults emerge from hibernation and fly about actively, seeking a place for oviposition. This is known as the spring flight and occurs in April and early May. Oviposition continues into June, reaching a maximum late in May. The majority of the larvae are found feeding on the alfalfa leaves in early June in Utah. They pupate in June, and the adults emerge in early July to feed and fly around for a month or so (summer flight), after which they appear to become much less active, finally hibernating in the fall. There is only one complete generation per annum. In Utah there is sometimes a partial second generation, but the larvae do not survive the winter. The larvae strip the leaves from the first crop of alfalfa, and after that is cut they keep down the sprouts of the second crop until late in June, so that this crop is greatly reduced, and the third crop is so late as to be of little value.

DIRECT RELATIONS OF CLIMATIC FACTORS TO THE LIFE HISTORY

TEMPERATURE

Reeves et al. (*9, p. 91*)[2] state: "There is no definite hibernation in this species. The adults are quiet when it is cold and active when it is warm . . ."

Titus (*14, p. 108*), says: "The time of entering hibernation varies greatly in different years and in different localities. If the summer is long, with warm, sunshiny weather throughout the latter part of the season, the weevils enter hibernation late and many of them perish before the summer ends. On the other hand, if the late

[1] Received for publication June 11, 1924; issued May, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 492.

summer is rather cool and cloudy, they will go into hibernation earlier, and apparently more of them pass through the winter successfully. In the higher mountain valleys the weevils become adult later in the summer and enter into hibernation earlier."

Apparently the rate of oviposition is closely related to temperature. Parks (*7*) has published detailed information upon the relation of temperature to oviposition, and it is evident from his chart (*7, p. 420*) that there is very little oviposition until the mean daily temperature reaches about 50° F. Titus (*14, p. 115*) makes the following statement, which is substantiated by Parks:

"The length of the egg-laying period each year naturally has a decided effect on the length of time that appreciable injury will occur to the crop for that year. Warm, dry, spring weather appears to be conducive to rapid egg laying, and thus many more larvae are feeding on the alfalfa at any one time than when the egg-laying period is extended over several months. Under these conditions more larvae come to maturity and the damage to the crop is more severe, since so many worms are present that there is little chance for the plant to recuperate. On the other hand, a slow cold spring and summer means that egg laying will continue for many weeks, and though probably as many larvae actually feed throughout the season, the damage to the crop harvested is not so great, the plant continually having opportunity to develop new buds and leaves. This readily explains the apparent decrease in injury in infested regions in 1912 as compared with the loss in 1911 in the same sections of the State."

The following table shows conditions in the spring months at Salt Lake City in 1911 and 1912:

Month	Mean temperature		Total precipitation	
	1911	1912	1911	1912
	°F.	°F.	*Inches*	*Inches*
March	44.4	40.0	2.04	3.48
April	47.9	46.8	1.65	2.34
May	57.2	55.8	1.84	1.75
Means and totals	49.8	47.5	5.53	7.57

These figures, which substantiate the above statement so far as climatic conditions are concerned, are taken from the Annual Meteorological Summary (*16*) for Salt Lake City for 1921.

The following notes regarding the influence of altitude upon damage are of particular interest when considering temperature.

Ball (*1, p. 144*): "As it passes out of the warm sections, around Utah and Salt Lake Valleys, its damage has become less and less until, in quite a number of districts, it is pretty hard to convince the farmers that they have it at all."

Titus (*15, pp. 133–134*): "In no place in Idaho is it known to be doing damage. Weevils are so few in most places that you have to search for them."

This statement was made in 1916, when the weevil was confined to the higher parts of southeastern Idaho.

Reeves et al. (*9, p. 88*): ". . . There is hope also that the pest will not prove equally injurious under all circumstances. It is much less harmful in Europe than in America, owing apparently to climatic and industrial conditions, and it multiplies more slowly and does less damage in the higher altitudes in Utah and Wyoming than in the lower valleys."

In considering the migrations of the adult beetles, the statements below may be quoted.

Reeves et al (*9*) *p. 87*): "There is no evidence that the weevils ever fly for the purpose of seeking fields of alfalfa, either new or previously infested, or to find hibernation quarters. The most plausible theory is that their flight is caused by a rise in temperature, as are many activities of the lower animals. So far as can be learned, this flight is at random. It takes some of the weevils into new fields." And, again (*9, pp. 92–93*): "The heat of the soil is also probably an important cause of that increase in the activity of the adults, called the summer flight, which is greatest during the dry, hot weather beginning in June and ending in August. This flight accounts for the presence of many adults in grassy places and orchards, where they alight and find protection from the heat . . . The summer flight is not a general movement of the weevils from the fields to seek more suitable hibernation places elsewhere. There is no such movement, and virtually all of the weevils spend the winter in the fields."

Very little was found regarding the *minimum* fatal temperature. The remark of Reeves (*9, p. 91*), quoted below, is of importance: "Many weevils die in the fields during zero weather, but milder temperatures seem to have little effect upon them."

One of the first successful control measures was based upon the *maximum* fatal temperature. In connection with a discussion of harrowing after removing the first crop Reeves (*8, p. 128*) states: " . . . Under such circumstances the insects can be destroyed and the crop protected by taking advantage of the fact that a temperature of 120° F. is fatal to the insects. This temperature is best produced by covering the surface of the field with something approaching a dust mulch, unshaded by clods and vegetation. On a bright, warm day such a surface is heated by the sun enough to kill all stages of the weevil, and the dust kills many of those which escape the heat."

The very high temperature obtained at the soil surface in direct sunlight has rarely been utilized to control insects.

HUMIDITY

In addition to those passages quoted above, in which reference is made to humidity in connection with temperature, there are a few places in which humidity seems to be more important than temperature.

Titus (*12, p. 49*) says: "During the period when they are going into hibernation they are quite susceptible to climatic conditions; even the passing of the sun behind a cloud will drive them to shelter, and a cold rain following a very hot day will so chill those that have not been able to get into proper shelter that many will not survive the night."

INDIRECT INFLUENCES OF CLIMATIC FACTORS

The parasites of the alfalfa weevil are of two kinds—insect parasites and fungi. The parasitic insects which have proven of importance in the control of *Phytonomus posticus* have been secured from Italy, a region of nearly optimum conditions for the weevil. On the other hand, fungi have been of importance in the Old World near the distributional limits. No information of value has been published regarding the climatic relations of insect parasites, but there are several statements concerning the fungi.

There are two entomophagous fungi reported as attacking the weevil. One of these, *Empusa sphaerosperma*, is an important enemy of a closely related species, the clover leaf-weevil (*Hypera punctata*), in the eastern United States and has killed as high as 44 per cent of the mature larvae of *Phytonomus posticus* at Salt Lake City. (*18, p. 41.*) Webster reports a mortality of about one-fifth on June 13, 1911, at Salt Lake City. The meteorological records show that the spring of 1911 at that place was nearly normal (see table on p. 480), which proves that this fungus is capable of destroying large numbers of the weevils under average Utah conditions and may prove of great importance near the limits of the range, where the spring conditions are cooler and wetter. A second species (*Sporotrichum globuliferum*) has been reported by Rockwood (*10, p. 499*) to be working on the adult beetles in Utah. Some of his conclusions are quoted below:

"The entomogenous fungus *Sporotrichum globuliferum* Speg. develops spontaneously as an infectious disease of the alfalfa weevil, *Hypera variabilis* (*posticus*), on the bench lands of the Salt Lake Valley in the early spring. Infection experiments show the weevil to be very susceptible to fungus infection at this season, a complete mortality from the fungus being secured in breeding cages in usually two weeks time. The ground-frequenting habits of the alfalfa weevil at this season render it particularly liable to infection from contact with fungus-covered insects.

"The new generation of weevils is less susceptible to the fungus during the periods of aestivation and hibernation in the summer and fall. Moreover, favorable conditions for the growth and spread of the fungus are unlikely to occur in Utah at this time.

"The period of greatest mortality from the fungous disease, coinciding as it does with a period of great potential injury from the pest, namely, the oviposition period, makes the fungus worthy of record as a natural enemy of the alfalfa weevil."

Some further remarks by Rockwood (*10, p. 493–494*) on the relations of climate to the abundance of Sporotrichum are relevant: "The fungus *Sporotrichum globuliferum* Speg. was first found on the alfalfa weevil near Salt Lake City, Utah, on March 14, 1914. It was frequently met with and could easily be found on weevils and other insects under alfalfa plants from that time until May. This was a time of considerable precipitation and the ground in alfalfa fields was moist to wet most of the time. The time of greatest abundance of the fungus was April 21 to 29. At this time at least one weevil killed by the fungus could be found under almost every plant examined . . . . . In the early spring, the optimum conditions for the growth of the fungus are likely to be found in all the alfalfa fields on the East Bench of old Lake Bonneville regardless of the irrigation practice.

"Later in the season when the spring rains have ceased, the fungus seems to be restricted to fields which are generously irrigated and have a heavy, close stand of alfalfa. One such field was examined on July 29 and 28 weevils with a pure growth of Sporotrichum were picked up in a short time. Such mortality at this late date is of slight importance, however, as this is the time when the overwintered adults are dying off naturally. Yet it is worthy of note that this field for which a generous water supply was available and which was therefore lavishly irrigated has never been seriously injured by the weevil, at least so the rancher informed me, and I was inclined to believe him, as it was certainly unusual for a Utah farmer to deny injury from the weevil . . ."

The above statements give a basis for determining the optimum conditions for fungous growth on the alfalfa weevil at Salt Lake City. The normal temperature for April is 50° F., and the normal April rainfall about 2 inches. So far as the production of a favorable humidity is concerned, this would be roughly equivalent to a monthly rainfall of 2½ inches at 60° F., or of 3 inches at 70° F. (*2, p. 65*). On this assumption, we can estimate the climatic suitability of any region for fungous growth by studying its approach to this curve, and we can tell whether or not the fungus *Sporotrichum globuliferum* will be an economic factor. This point is used frequently in studing the distribution of the alfalfa weevil in America.

Summary of Climatic Relations of Phytonomus Posticus in America

The following points of importance in this study have been found in this survey of American literature:

The limiting temperatures are about 0° F. and 120° F.

Warm, dry spring weather is essential for the rapid multiplication of the weevil. Such weather shortens the season of oviposition and produces a maximum number of larvae working simultaneously.

The damage decreases with an increase in altitude. An increase in altitude in this region is equivalent to a shortening of the growing season plus an increase in precipitation.

Cold, damp spring weather like that of Montana, as opposed to the hot, dry spring weather of the Great Basin, not only retards the rate of development of the weevil itself, but is favorable to the increase of fungous enemies, which aid in checking the damage.

THE CLIMATIC ECOLOGY OF PHYTONOMUS POSTICUS IN THE OLD WORLD

The Old World distribution of the alfalfa weevil is given by Titus (*13*) as all of Europe, southern Siberia, Turkestan, Asia Minor, Persia, Arabia, north coast of Africa, Madeira, and Canary Islands. He records outbreaks of this or some very closely related species in southern France, Italy, and southern Russia.

This is a very general statement from which to develop the climatic relations of this insect, so the writer has used a second method of determining the economic distribution. A search was instituted through two abstracting periodicals, the Review of Applied Entomology, Series A, and the International Review of the Science and Practice of Agriculture. The material is presented below in three categories. "Mention" indicates a mere mention of the insect in a general list of pests, with nothing to show that any damage was done by it. "Minor pest" indicates that some slight damage was done in the year indicated. "Major pest" indicates a serious outbreak, recorded in one or several extended articles. Many of the articles recorded below are not important in the further study of the weevil, so the original sources are not quoted.

Economic Distribution of P. Posticus

MAJOR PEST:

Southern France.—1914. Controlled to some extent by a fungus, *Entomophthora sphaerosperma.*

Italy.—No recent references. Titus (*13*) gives the following dates of outbreaks: 1884, 1890, 1909, 1910, 1911.

Turkestan.—1913. In connection with a paper on the utility of the Wagtail, an insectivorous bird, the alfalfa weevil is noted as a very serious pest. It does a large amount of damage, there being no natural control for it except birds.

———. 1914. Listed as a major pest in the valley of Isfara (Ferghana) this year.

MINOR PEST:

Sweden.—1912.

Denmark.—1916. Districts of Lolland-Forster and Naestvod, controlled by fungus.

Germany.—1922.

Southern Russia.—Governments of Kiev, Moscow, and Kherson. No dates given.

Transcaucasia.—Districts of Tiflis and Erivan. Was a pest in these regions in 1916–17.

MENTION:

Denmark.—1918, 1919, 1920.

Russia.—Moscow. No dates given.

Astrachan.—Mentioned in a list of pests of alfalfa.

Reeves (*8, p. 130*) makes the following statement, which explains the scarcity of serious outbreaks in Italy. . . . "There is a possibility that in a different climate from that of Utah

the weevils would not be forced into inactivity by the heat of summer, the generations would be more or less spread out, and the feeding would continue through a larger portion of the year but be less concentrated and therefore less destructive. . . . Mr. H. S. Smith, who studied the insect in Italy, was of the opinion that that was one of the principal factors in producing the condition in Italy along the seacoast, where the weevil is always present in considerable numbers, but is of no consequence as a pest. Its feeding is distributed through many months instead of a few weeks."

Summarizing the above information: The alfalfa weevil is at times a major pest in southern France, Italy, and Turkestan; a minor pest in Germany, Sweden, Denmark, southern Russia, and Transcaucasia. If that gives an approximate picture of the economic distribution of this species, the climatic optima are found in the Mediterranean region and Turkestan and the limiting conditions farther north, in Germany, Sweden, Denmark, and southern Russia, with a limiting condition of a similar nature in Transcaucasia.

Climatic Conditions

The writer has spent considerable time collecting and comparing the climatic data for the various regions listed above, and is greatly indebted to C. F. Talman, librarian of the United States Weather Bureau at Washington, for securing data and for other services in this connection. The basic data have been taken from Hann (*3*) and Kendrew (*4*), and it is impracticable to cite specific references for each quotation. The climatic data have been very carefully studied, and no attempt is made to give those for any given station in full, such a citation being replaced by a summary of the important conditions of a general region as shown by a study of several stations.

Optimum Conditions

Mediterranean region.—The general character of the annual cycle of temperature and precipitation is well shown in the climograph for Marseilles, France (fig. 1). The climograph of Ball is a graph on which monthly means of temperature are plotted against monthly totals of precipitation. The points are joined in chronological order, so that a picture of the annual cycle of temperature and rainfall is obtained. All the stations studied in southern France and Italy have essentially similar climates, so that the region may be treated as a unit. The important factors in this study are (1) the warm, wet winters, no month having a mean temperature below freezing, and (2) the hot, dry summers, seven to nine months being above 50° F. and five to six months above 60° F. in mean temperature. The spring from April to July is increasingly dry, March and April being the only months favorable for fungous growth. The precipitation of the three summer months—June, July, and August—ranges from 2½ to 4 inches for the period, averaging very close to 3 inches. The mean relative humidity in summer ranges from 50 to 65 per cent, and all times of the year are very sunny, most of the winter rainfall coming in heavy showers, followed by bright sunshine.

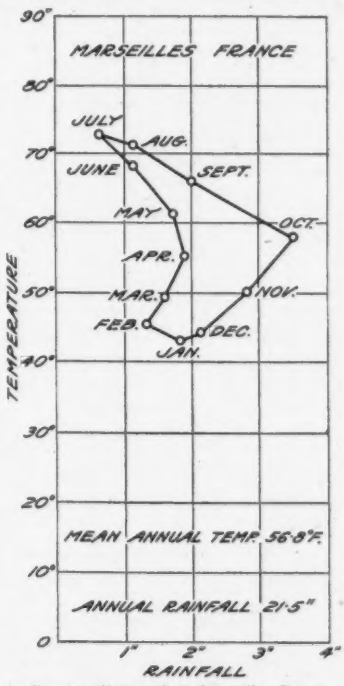


Fig. 1.—Climograph for Marseilles, France

Turkestan.—The attacks of the weevil in this region have been most severe in the Valley of Ferghana. This is an irrigated valley in the mountains of the southern part of the province, which has climatic conditions very comparable to those of

the Salt Lake Valley in Utah, and for that reason a study of its climate is of considerable interest.

At the station of Tashkent (fig. 2) a mean annual temperature of 56° F. is found, with five months above 60° F. and seven months above 50° F. The mean annual maximum is 103° F. and the mean annual minimum −4° F., the absolute minimum for 30 years being −19° F. The rainfall comes largely in early spring, March and April being the wettest months, with conditions very favorable for fungous growths. The spring is increasingly dry until July, and the total rainfall for the summer months is only 1 inch. To judge from the summer temperatures, the frostless season must be well over 150 days. The relative humidity is 63 per cent for the annual mean and 48 per cent in July. There is sufficient winter precipitation to provide a snow cover at the times of subzero temperatures.

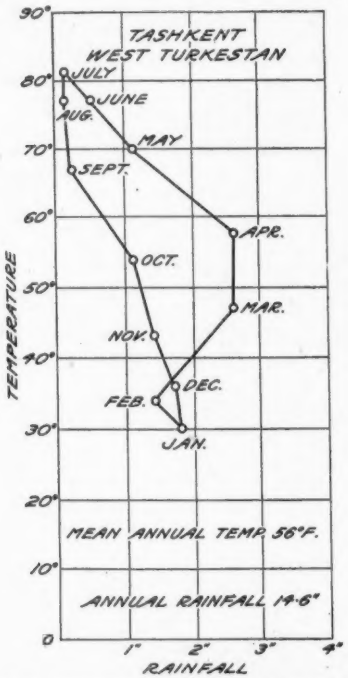


FIG. 2.—Climograph for Tashkent, West Turkestan

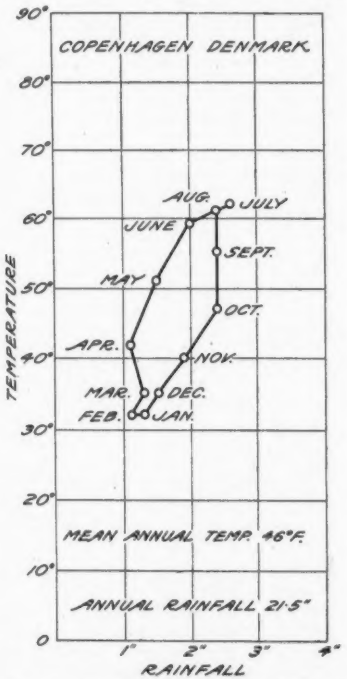


FIG. 3.—Climograph for Copenhagen, Denmark

LIMITING CONDITIONS

SCANDINAVIAN COUNTRIES.—Copenhagen, Denmark (fig. 3), has been chosen as typical of conditions in this general region. The climate is maritime, with fairly evenly distributed rainfall. The spring is the driest part of the year, with the rainfall gradually increasing to a maximum in July, when conditions become favorable for fungous growth on the alfalfa weevil. The summer, like the spring, is cool, so that feeding by the weevil must be scattered over a long time. A reference in the Review of Applied Entomology notes larval feeding in July (*6*). This would indicate that growth is retarded. Fungous enemies are also noted. Thus the limiting conditions in this region are cool summers and too abundant moisture.

SOUTHERN RUSSIA.—Of the three governments listed in the table, that of Moscow is farthest north and should furnish a better index of limiting conditions than either of the others. The data from several stations in Kiev and Kherson indicate that heavy spring rainfall is the limiting factor. The climograph for Moscow (fig. 4) shows a climate quite similar to that of our western plains, although the winter

minimum of rainfall is not so strongly marked. The limiting factors appear to be summer rainfall, which averages about 7.6 inches, and winter temperatures. The mean annual minimum temperature is −23° F., and the five winter months average below the freezing point. The growing season is about 165 days. The spring is slow, so that feeding is distributed over a long period.

ASTRACHAN.—The climograph for Astrachan is shown in Figure 5. The climate is warmer than that of Moscow, and the rainfall at all times is very light, so that the limiting factor in this case must be winter temperature. The mean annual minimum is −13° F., and what little snowfall there is must be swept away by the winds, so that the weevils are kept down by the cold winters. Spring and summer conditions of temperature and precipitation are close to the optimum, being warm and dry.

TRANSCAUCASIA.—This is a mountain region of extremely varied climate. Data are available from the two stations of Tiflis and Erivan, and their climographs are reproduced in Figures 6 and 7. Tiflis has warmer winters than Erivan, but Erivan has drier summers. The limiting condition at Tiflis is apparently the precipitation in the spring months, May, especially, being favorable to fungous enemies. At Erivan summer conditions approach more closely to those of the Mediterranean region, but the winters are cold.

No data are available regarding minimum temperatures at Erivan, but the absolute minimum at Tiflis is 1° F., and the winter months at Erivan average 10° F. colder than at Tiflis, so that subzero temperatures must be fairly common. Of the two regions, that around Erivan approaches the rainfall optimum, whereas Tiflis presents a limiting condition of rainfall.

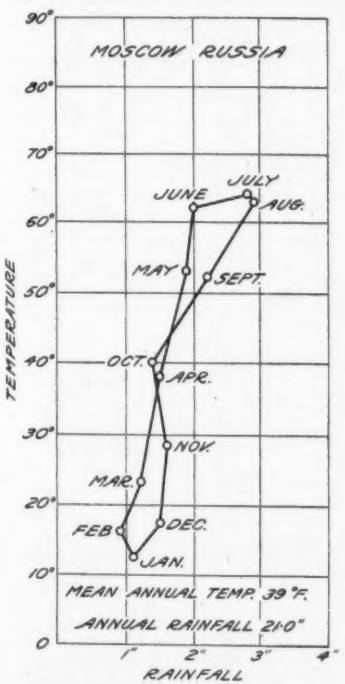


FIG. 4.—Climograph for Moscow, Russia

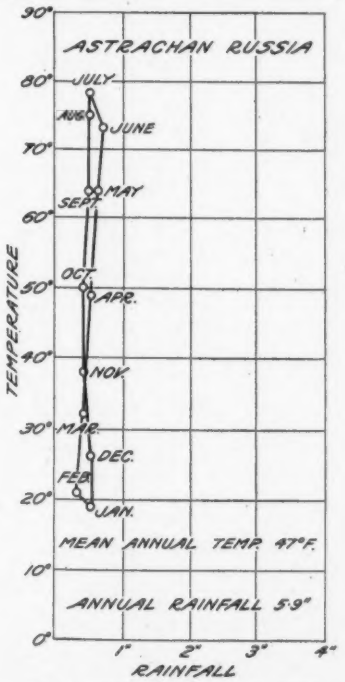


FIG. 5.—Climograph for Astrachan, Russia

SUMMARY OF THE CLIMATIC RELATIONS OF PHYTONOMUS POSTICUS IN THE OLD WORLD

The climatic requirements of the alfalfa weevil in the Old World will

here be summarized for comparison with those requirements in America.

One of the limiting conditions is winter temperature. The insects are killed by exposure to 0° F., and places which normally have subzero temperatures, not accompanied by heavy snow covering, are shown to be found on the limits of distribution. (Moscow, Astrachan.)

Warm, dry spring weather is shown to be essential to the rapid development of the weevil. Such conditions obtain in southern France, Italy, and Turkestan, and to a lesser extent in Tiflis. The spring must be increasingly dry, and the summer precipitation must be not more than 4 inches. Each of the months whose mean temperature is above 50° F. should have less than 2 inches of rainfall.

Damage decreases with an increase in altitude. This is climatically equivalent to an increase in latitude. The northern boundary is found in regions having a growing season of about 150 days, with a mean annual temperature of about 40° F., four months above 50° F. and three months above 60° F.

Cold, damp spring weather favors the increase of fungous enemies. Such weather is found in Denmark, southern Russia, and to a lesser extent in Transcaucasia. Months whose mean temperature is above 50° F. have more than 2 inches of rainfall.

No data are available from which to determine the factors which limit distribution on the south.

For the purpose of applying these findings to American conditions they are condensed into the following conditions:

(*A*) Optima:
- Mean annual temperature 50° F. or above.
- Summer rainfall not over 4 inches.
- No winter temperatures below 0° F. or, if below, in combination with heavy snowfall.
- Growing season of 150 days or more.

(*B*) Limits:
- Mean annual temperature below 40° F.
- Summer rainfall over 6 inches.
- Subzero winter temperatures combined with light snowfall.
- Growing season less than 100 days.

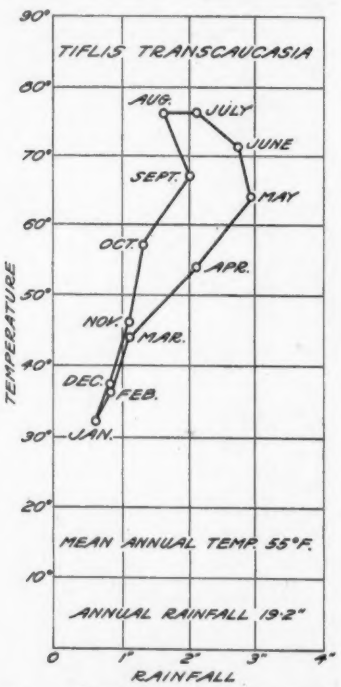


FIG. 6.—Climograph for Tiflis, Transcaucasia

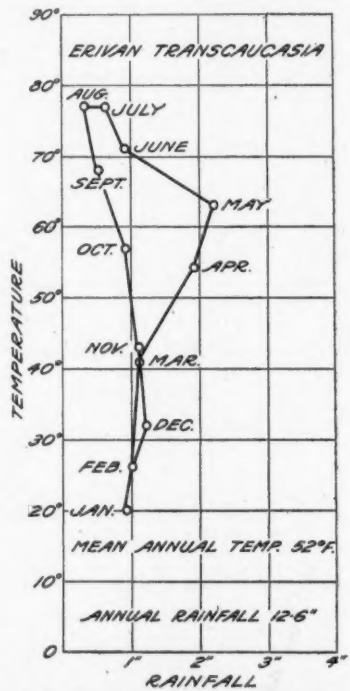


FIG. 7.—Climograph for Erivan, Transcaucasia

SUITABILITY OF AMERICAN CLIMATE FOR THE ALFALFA WEEVIL

RAINFALL

Students of American climatology recognize several types of rainfall distribution. Ward (*17*) lists 14 rainfall types, but for our purpose there are 6 main types, as designated by Kincer (*5*), which he calls the Florida, Eastern, Plains, Arizona, sub-Pacific, and Pacific types.

Of these 6 types the summer rainfall is less than 6 inches in four types and less than 4 inches in two of these. The Plains type is characterized by a summer maximum, the summer rainfall ranging up to 12 inches or more. However, there are certain parts of the plains, on the eastern slope of the Rockies, in which the summer rainfall is from 4 to 6 inches, and it is in these places that the weevil may possibly occur. The Arizona rainfall type is characterized by a late summer maximum in July and August, but there are some regions in this type in which the summer rainfall is very small. The Pacific type has a very pronounced winter maximum, with almost rainless summers. The sub-Pacific type, illustrated by Salt Lake City (fig. 8), has no pronounced winter maximum, but fairly heavy rainfall occurs from March to May. Farther north in Idaho there is a more pronounced winter maximum, with a secondary maximum in May. (Payette, fig. 9.) In western Colorado the rainfall is very light with no pronounced maximum. (Delta, fig. 10.)

The weevil is apparently excluded from most of America east of the Rocky Mountains by abundant spring and summer rainfall. It would appear to be limited to such districts west of the Rockies as have favorable temperature conditions and to a small area lying immediately east of the Rockies, which has scanty summer rainfall.

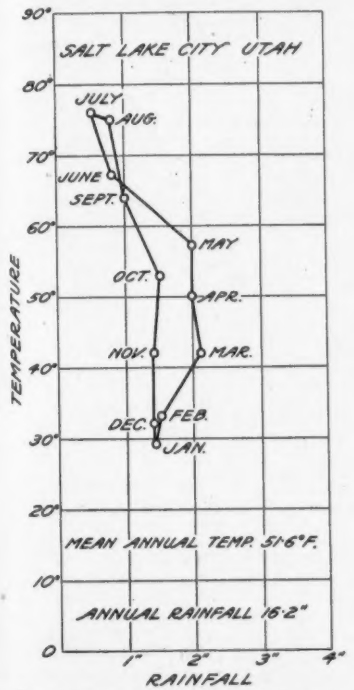


FIG. 8.—Climograph for Salt Lake City, Utah

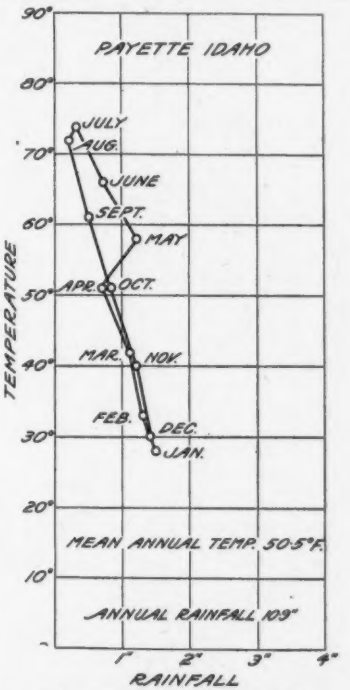


FIG. 9.—Climograph for Payette, Idaho

TEMPERATURE

Within the rainfall regions outlined above the distribution and abundance of the weevil are controlled by temperature. Regions having a mean annual temperature of 50° F. or higher approach the optimum. When this tem-

perature is approached, although winters are cold, the weevil may maintain itself and become of economic importance periodically. A mean annual temperature of 40° F. closely marks the northern limit of economic distribution.

Probable Distribution in America, Based on Climate

The writer carefully analyzed the climatological data from the Western States, considering all the possible

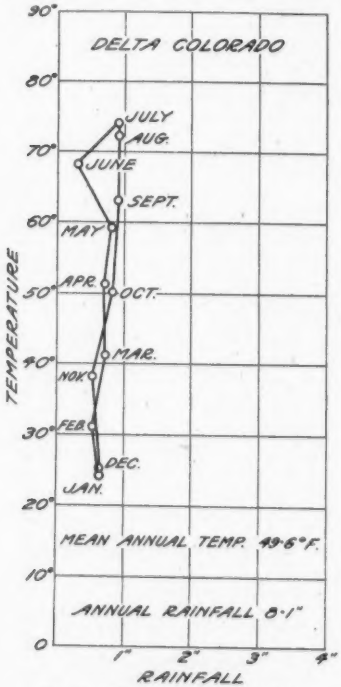


FIG. 10.—Climograph for Delta, Colo.

combinations of temperature and rainfall found to be of importance in the ecology of the alfalfa weevil. The stations may be tentatively grouped under three categories:

1. Regions whose normal climate approaches the optimum for the alfalfa weevil may be regarded as subject to severe infestation and included in an area of "normal" occurrence.

2. Regions whose normal climate departs slightly from the optimum may be regarded as subject to periodic infestation in times when the climatic variations are toward the optimum. These regions are areas of "occasional" occurrence. The weevil is able to maintain itself at all times in these areas and becomes abundant when conditions become favorable.

3. Regions whose normal climate varies widely from the optimum, in which optimum conditions will rarely occur, may be included in an area of "possible" occurrence. The weevil will probably not maintain itself in this area but may be repeatedly introduced and become of minor importance after a series of favorable seasons.

The probable distribution of the alfalfa weevil in America, based upon these climatic requirements, is shown in the map (fig. 11). The normal occurrence of the weevil is limited entirely to the sub-Pacific and Pacific rainfall regions, which are indicated on the map. There are two localities outside of these regions which are included in the second zone, one in northern Wyoming and the other in western New Mexico. Rainfall conditions are favorable in Wyoming, but the mean temperature is low and the winters are cold and dry; therefore this area is not of great importance. Western New Mexico, on the other hand, has favorable temperature conditions, but the rainfall is of such a character that wet years are very likely to occur often enough to keep the insect in control. The possible occurrence of the outer zone, which are confined to the Plains and Arizona rainfall types are chiefly conditions comparable to Moscow, Russia (fig. 4), but with more extreme winter conditions.

Broadly speaking, the alfalfa weevil is limited on the north, inside the Pacific and sub-Pacific rainfall regions, by low summer temperatures; on the east, at the edge of the sub-Pacific type, by heavy summer rainfall; and on the northeast, in the plains of Montana and Wyoming, by the summer rainfall and by low winter temperatures, plus a very light snowfall.

No data have been found upon which to base a limit on the south, but it is possible that the high temperatures of southern California and Arizona may prove fatal to larvae. All of this region is included in the normal zone in this study, and must remain so until the factor controlling southern distribution becomes evident.

The question of the possible adaptation of the alfalfa weevil to new climatic conditions in America has not been approached in this paper and does not seem of importance to the writer. The weevil is a native of Europe and

has been limited on the north by purely climatic factors for a long period of time. There is no physiographic barrier at its northern limit in Russia and Siberia, and it seems probable that it has reached its limit of adaptability by the present time, so that little or no further adaptation to cold and moisture will take place.

persal of the weevil by approximately five-year periods. This map does not show the present distribution accurately, as a county has been recorded as infested when weevils have been found in any portion of it. The infestation in southern Wyoming, for example, does not cover more than a third of the area shown in Figure 12.

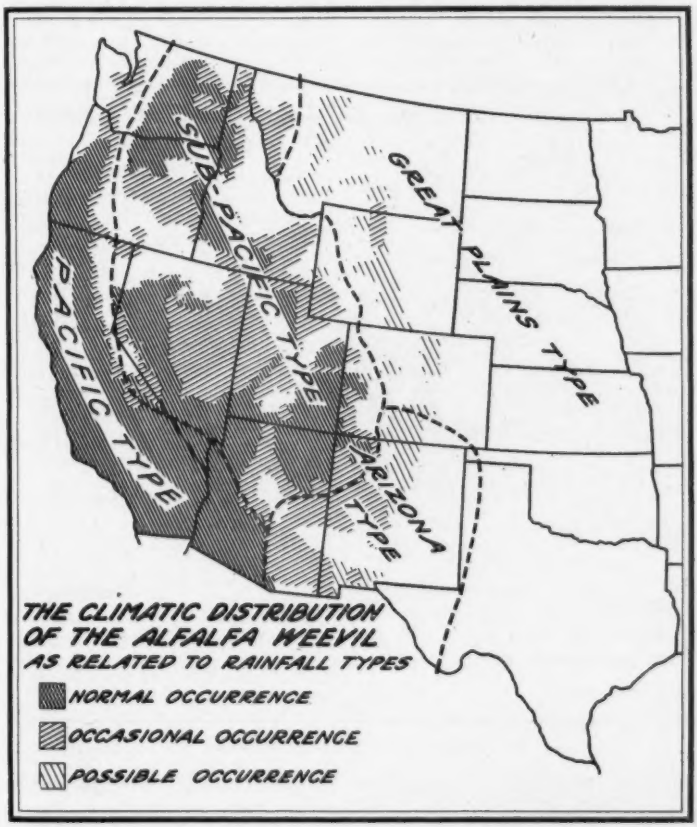


FIG. 11.—Probable distribution of the alfalfa weevil in America, based on climatic limitations

PRESENT DISTRIBUTION

Through the kindness of George I. Reeves, of the United States Bureau of Entomology, the writer obtained a detailed statement of the annual spread of the alfalfa weevil by counties since its introduction and discovery at Salt Lake City in 1904. This information has been summarized and a map prepared (fig. 12), which shows the dis-

This map brings out several interesting facts. All regions infested at the present time are within the rainfall area of the sub-Pacific type. The weevil is still spreading northward and westward in Idaho and Oregon and southward in Utah. On the other hand, it reached its climatic limits in Colorado in 1917, and its dispersion has been only local in that region since that time. It has not crossed the

Continental Divide there. It has been present in southwestern Wyoming for eight years and has not crossed the climatic limit shown on the map. It reached the northeastern corner of Idaho in 1917, and has not yet crossed the climatic boundary into Montana.

Within the present range the regions of the most severe infestation are the lower valleys of Utah, the Colorado area, western Idaho and the adjacent part of Oregon, and western Nevada. It has been abundant in some of the other places, but varies greatly in abundance from year to year. In short, it is most abundant in the zone of "normal" occurrence, is often injurious in the zone of "occasional" occurrence, but has not as yet reached the zone of "possible" occurrence. It is spreading at the present time in those parts of its range where conditions are favorable from a climatic standpoint and has ceased to spread when it has reached the climatic boundaries. Thus the history of the insect in America substantiates the findings of the studies on climatic conditions in the Old World.

The zones outlined on the map of Figure 11 are based upon a detailed study of the climatological data from several hundred stations in this region as published by the United States Weather Bureau.[3] The writer wishes to express appreciation of the assistance rendered by the section directors of the Weather Bureau for these Western States in furnishing a large amount of climatic data and in some cases lending file copies of the older publications.

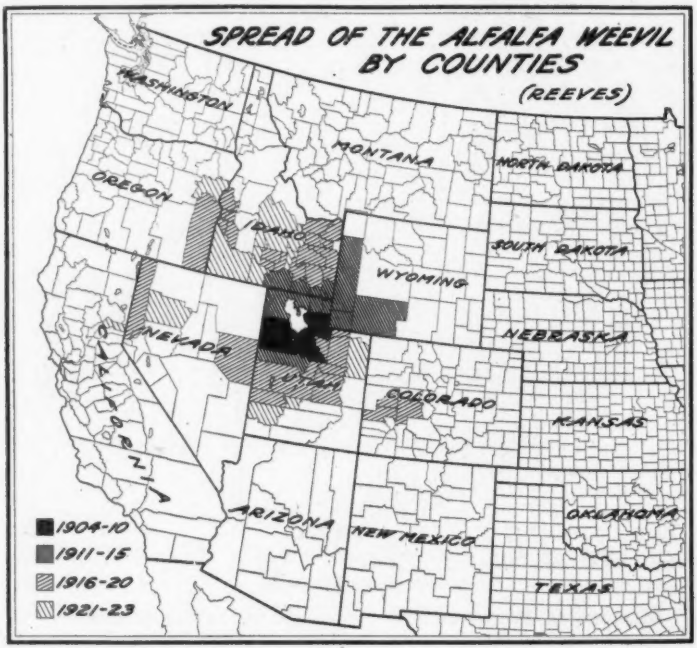


FIG. 12.—Dispersion of the alfalfa weevil in America, 1904–1923

SUMMARY AND CONCLUSIONS

The general procedure indicated in the introduction has been used in the present paper.

The climatic conditions of the original home of *Phytonomus posticus* have been studied in detail to find the optimum and limiting conditions, which were correlated with similar conditions in America.

[3] UNITED STATES DEPARTMENT OF AGRICULTURE, WEATHER BUREAU. SUMMARY OF THE CLIMATOLOGICAL DATA FOR THE UNITED STATES, BY SECTIONS. 106 sections, published separately. 1908 and later dates.

UNITED STATES DEPARTMENT OF AGRICULTURE, WEATHER BUREAU. CLIMATOLOGICAL DATA BY SECTIONS. Annual summaries under this general title for various Western States. 1910–1922.

Climatic conditions in America were analyzed to determine the areas of optimum and limiting conditions, which have been charted and compared with the present distribution of the alfalfa weevil. They have been found to correspond in all essential particulars with the progress of the present infestation.

It seems highly probable that the alfalfa weevil has been imported into this country many times and that it also has often been shipped out of the quarantined areas into other parts of the country, but that it has failed to establish itself except where it was placed in a region of favorable climatic conditions.

The climatic limitations of other insects are different, for every insect has its own definite optimum and limiting factors. Some insects, like the San Jose scale and the brown-tail moth (*11*) are definitely limited on the north by minimum temperatures. Others require a certain length of growing season. Still others, chiefly boreal forms, can endure intense cold but succumb to heat and have a definite southern limit. With regard to rainfall, the same is true. Insects like the army worm and the variegated cutworm require a humid condition, and others like the pale western cutworm require a dry condition. It seems highly probable that there are one or more periods in the life of any insect when it is especially susceptible to unfavorable climatic conditions. If these periods can be found, and their climatic optima and limits determined, the range of the insect can be mapped. In the case of the alfalfa weevil the limiting periods are two in number—the temperature limit, which applies largely to the hibernating adults, and the humidity limit, which applies to the larva and its fungous enemies.

The method of climatic analysis used in this paper has been applied successfully to an insect inhabiting the soil (the pale western cutworm (*2*)) and to an insect feeding on the aerial portions of low plants (the alfalfa weevil). In order to determine the applicability of the method to insects in general, it remains to apply it to some insect living in trees. The forest environment, either natural or in a cultivated orchard or grove, is widely different from conditions in the open, and the problem must be attacked by an analysis of actual forest conditions, after which these conditions must be correlated with the available weather data from near-by stations. For this reason the method will probably need considerable modification before it will apply to forest insects.

LITERATURE CITED

(1) BALL, E. D.
1917. [DISTRIBUTION OF ALFALFA WEEVIL IN UTAH]. Bien. Rpt. State Hort. Com. Utah 1914–16: 144–145.

(2) COOK, W. C.
1924. THE DISTRIBUTION OF THE PALE WESTERN CUTWORM, POROSAGROTIS ORTHOGONIA MORR: A STUDY IN PHYSICAL ECOLOGY. Ecology 5: 60–69, illus.

(3) HANN, J.
1908–11. HANDBUCHDER KLIMATOLOGIE. Aufl. 3, 3 Bd., illus. Stuttgart.

(4) KENDREW, W. G.
1922. THE CLIMATES OF THE CONTINENTS. 387 p., illus. Oxford.

(5) KINCER, J. B.
1919. THE SEASONAL DISTRIBUTION OF PRECIPITATION AND ITS FREQUENCY AND INTENSITY IN THE UNITED STATES. Mo. Weather Rev. 47: 624–631, illus.

(6) LIND, J., ROSTRUP, S., and RAVN, F. K.
1919. [REPORT ON AGRICULTURAL PESTS IN DENMARK IN 1916] (Abstract) Rev. Appl. Ent. (A) 7: 446–447.

(7) PARKS, T. H.
1914. EFFECT OF TEMPERATURE UPON THE OVIPOSITION OF THE ALFALFA WEEVIL (PHYTONOMUS POSTICUS GYLLENHAL). Jour. Econ. Ent. 7: 417–421, illus.

(8) REEVES, G. I.
1917. THE ALFALFA WEEVIL INVESTIGATION. Jour. Econ. Ent. 10: 123–130.

(9) ——— and others.
1919. THE ALFALFA WEEVIL AND METHODS OF CONTROLLING IT. Bien. Rpt. Bd. Hort. Oreg. 15: 83–98, illus.

(10) ROCKWOOD, L. P.
1916. SPOROTRICHUM GLOBULIFERUM SPEG., A NATURAL ENEMY OF THE ALFALFA WEEVIL. Jour. Econ. Ent. 9: 493–499.

(11) SANDERSON, E. D.
1908. THE INFLUENCE OF MINIMUM TEMPERATURES IN LIMITING THE NORTHERN DISTRIBUTION OF INSECTS. Jour. Econ. Ent. 1: 245–262, illus.

(12) TITUS, E. G.
1910. THE ALFALFA LEAF-WEEVIL. Utah Agr. Exp. Sta. Bul. 110, 72 p., illus.

(13) ———
1911. THE GENERA HYPERA AND PHYTONOMUS (COLEOPTERA, FAMILY CURCULIONIDAE) IN AMERICA, NORTH OF MEXICO. Ann. Ent. Soc. Amer. 4: 383–473, illus.

(14) ———
1913. THE CONTROL OF THE ALFALFA WEEVIL. Utah Agr. Exp. Sta. Circ. 10: 105–120, illus.

(15) ———
1917. [PRESENT DISTRIBUTION OF ALFALFA WEEVIL] Bien. Rpt. State Hort. Com. Utah 1914/16: 133–134.

(16) UNITED STATES DEPARTMENT OF AGRICULTURE, WEATHER BUREAU.
1921. ANNUAL METEOROLOGICAL SUMMARY WITH COMPARATIVE DATA FOR SALT LAKE CITY, UTAH.

(17) Ward, R. D.
1917. RAINFALL TYPES OF THE UNITED STATES. Geogr. Rev. 4: 131–144, illus.

(18) WEBSTER, F. M.
1912. PRELIMINARY REPORT ON THE ALFALFA WEEVIL. U. S. Dept. Agr., Bur. Ent. Bul. 112, 47 p., illus.